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(54) Title: ALZHEIMER'S DISEASE THERAPEUTICS

(57) Abstract

A method of identifying a therapeutic useful for treating or preventing Alzheimer's disease, which method includes the steps of contacting (a) a first molecule containing the couplone portion of APP (SEQ ID NO: 1) with (b) a second molecule containing the amino acid sequence of G_o (SEQ ID NO: 2) or an APP-associating region of G_o (SEQ ID NOs: 3, 4, or 5), in the presence of a candidate compound; and determining whether the candidate compound interferes with the association of the first and second molecules, such interference being an indication that the candidate compound is a potential Alzheimer's disease therapeutic.

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ALZHEIMER'S DISEASE THERAPEUTICS

The field of the invention is Alzheimer's disease therapeutics.

Background of the Invention

Alzheimer's disease (AD) is a progressive degenerative disorder of the brain that afflicts over four million people in the United States. No effective treatment is available. The most characteristic change observed upon post-mortem histopathological analysis of AD-afflicted brain tissue is the presence of neuritic and cerebrovascular plaques containing dense deposits of β-amyloid protein (Selkoe, Cell 58:611-612, 1989). β-amyloid is a 39-43 amino acid peptide (Glenner and Wong, biochem. biophys. Res. Commun. 120:885-890, 1984; Masters et al., Proc. Natl. Acad. Aci. USA 82:4345-4249, 1985) synthesized as part of a larger precursor protein referred to as amyloid precursor protein (APP), which is known to have a number of isoforms in humans (APP_{695}, Kang et al., Nature 325:733-736, 1987; APP_{751}, Ponte et al., Nature 331:525-527, 1988, and Tanzi et al., Nature 331:528-530, 1988; and APP_{770}, Kitaguchi et al., Nature 331:530-532, 1988). The amino terminal of β-amyloid is generated by cleavage of a peptide bond of APP which in APP_{695} lies between Met596 and Asp597.

Although structural alterations of APP are implicated in the pathogenesis of Alzheimer's disease, it remains unknown how they cause the disease. No biological function for APP has been identified, although there is evidence that APP has a receptor-like architecture (Kang et al., Nature 325:733-736, 1987; Ponte et al., Nature 331:525-527, 1988; Tanzi et al., Nature 331:528-530, 1988; Kitaguchi et al., Nature 331:530-532, 1988), is located on the neuronal surface (Dyrks et al., EMBO J. 7:949-957, 1988), and possesses an

evolutionarily conserved cytoplasmic domain (Yamada et al., Biochem. Biophys. Res. Commun. 149:665-671, 1987).

Summary of the Invention

The methods and therapeutical compositions of the invention are based upon the discovery, described in detail below, that APP forms a complex with G_o, a major GTP-binding protein (or "G protein") in brain. Like all G proteins, a molecule of G_o is made up of one α subunit and one $\beta\gamma$ subunit. Two isoforms of G_o, known as G_{o1} (or G_{oA}) and G_{o2} (or G_{oB}), have been identified; they have slight amino acid differences in their α subunits, and are together referred to herein as G_o. The cDNA sequence and deduced amino acid sequence of the α subunits of each of G_{o1} and G_{o2} (as reported by Strathmann et al., Proc. Natl. Acad. Sci. USA 87:6477-6481, 1990) are shown in Fig. 4a (SEQ ID NO: 2) and Fig. 4b (SEQ ID NO: 28), respectively.

The finding that APP associates with G_o is consistent with related findings concerning other G proteins, as disclosed in a second application (USSN________________) having the same inventor and filing date as the present application, which second application is herein incorporated by reference. The cytoplasmic APP_{695} sequence His^{657}-Lys^{676} (SEQ ID NO: 1) possesses a specific G_o-activating function, and is necessary for complex formation of this APP with G_o; this sequence, sometimes referred to as the "couplone" region of APP, is completely conserved in APP_{751} and APP_{770}, as well as in mouse APP_{695}. This provides evidence that APP is a receptor coupled to G_o, and suggests that abnormal APP-G_o signalling is involved in the Alzheimer's disease process.

The invention includes a method of identifying a therapeutic useful for treating or preventing Alzheimer's disease, which method includes the steps of

contacting (a) a first molecule containing the couplone portion of APP (SEQ ID NO: 1) with (b) a second molecule containing the amino acid sequence of G_o (SEQ ID NO: 2) or an APP-associating region of G_o (SEQ ID NOs: 3, 4, or 5), in the presence of a candidate compound; and

either (i) determining whether the candidate compound interferes with (i.e., inhibits partially or completely) the association of the first and second molecules, or (ii) determining whether the candidate compound interferes with the activation of the second molecule by the first molecule, such interference being an indication that the candidate compound is a potential therapeutic useful for treating or preventing Alzheimer's disease. The determining step may be accomplished by, for example, immmunoprecipitating the first molecule with an antibody specific for APP, and detecting the presence or amount of the second molecule which co-precipitates with the first molecule. Alternatively, the second molecule can be immunoprecipitated with an antibody specific for G_o, following which the presence or amount of the first molecule which co-precipitates with the second molecule is determined. Where activation is the criterion being measured, the determination step may be accomplished by contacting the second molecule with a substrate which is or includes GTP or an analog of GTP [such as GTPγS or Gpp(NH)p], and detecting or measuring the binding of the substrate to the second molecule, wherein such binding is evidence of activation of the second molecule by the first molecule. In preferred embodiments, the contacting step is carried out in a cell-free system; the Mg^{2+} concentration at which the contacting step is carried out is between approximately

$1x10^{-7}$ and $1x10^{-2}$ M, and the first molecule includes the cytoplasmic tail portion of APP_{695} from residues 649 to 695 (SEQ ID NO: 6) and/or the membrane-spanning portion of APP_{695} from residues 639 to 648 (SEQ ID NO: 7) (the entire membrane-spanning segment of APP_{695} being from residues 625 to 648, SEQ ID NO: 8); the first molecule more preferably includes substantially all of APP (SEQ ID NO: 9). (Alternatively, the corresponding functional regions of APP_{751} or APP_{770}, or any other APP, may be used.) The second molecule preferably contains two or three of the putative APP-associating regions referred to above, and may also contain one or more of the GTP-binding regions of G_o, corresponding to residues 35 to 50 (SEQ ID NO: 10), residues 201 to 218 (SEQ ID NO: 29), or residues 263 to 274 (SEQ ID NO: 30) of G_{o1} [Kaziro, "Structure of the genes coding for the α subunits of G proteins", Ch. 1 in ADP-ribosylating Toxins and G proteins (Moss, J., and Vaughan, M. eds.) pp189-206, American society for Microbiology, Washington, D.C. (1988)], and more preferably contains substantially all of G_o (SEQ ID NO: 2).

The invention also includes a system (e.g., a cell-free *in vitro* system) for screening candidate Alzheimer's disease therapeutics, which system includes a first polypeptide containing a sequence essentially identical to that of peptide 20 (SEQ ID NO: 1), and a second polypeptide containing a sequence essentially identical to one, two or three of the putative APP-associating regions of G_o (SEQ ID NOs: 3, 4, and 5); the system may also include a means for detecting either (a) the association of the first polypeptide with the second polypeptide, or (b) the activation of the second polypeptide by the first polypeptide. The first polypeptide may conveniently be anchored to a solid material (e.g., a cellular membrane, a polystyrene

surface, or a standard matrix material), or may be in a phospholipid vesicle. It may include a sequence essentially identical to the membrane-spanning region of APP, and/or a sequence essentially identical to the entire cytoplasmic tail of APP. The second molecule preferably contains the GTP-binding domain of G_o, and more preferably contains the entire sequence of G_o.

The invention also features a method for diminishing the activation of G_o in a neuronal cell by treating the cell with a compound, such as a peptide fragment of G_o or of the cytoplasmic tail of APP, which blocks association of neuronal G_o with, and/or activation of neuronal G_o by, the cytoplasmic tail of APP. The cell may be so treated *in vivo* (i.e., in an animal, e.g. a mammal such as a human or other primate, cow, horse, pig, sheep, goat, dog, cat, rat, mouse, guinea pig, hamster, or rabbit) or *in vitro*. This method may be used to prevent or treat the symptoms of Alzheimer's disease in a patient. Such a compound may include, for example, a peptide having fewer than 50 amino acids (preferably 40 or fewer, and more preferably 30 or fewer), and containing the sequence of peptide 20. Also within the invention is a DNA molecule (e.g., a plasmid or viral DNA) encoding such a peptide, and a therapeutic composition containing, in a pharmaceutically acceptable carrier, either the peptide or the DNA molecule.

In another aspect, the invention features a method for identifying a ligand for which APP is a receptor, which method includes the steps of

providing an APP molecule, the cytoplasmic tail of which is accessible to a molecule of G_o;

contacting a candidate compound with the extracellular domain of the APP molecule; and

detecting either (a) association of G_o with the APP molecule, (b) dissociation of G_o from the APP

molecule, or (c) activation of G_o by the APP molecule, such association, dissociation, or activation being evidence that the candidate compound is a ligand of APP.

Other features and advantages of the invention will be apparent from the detailed description set forth below, and from the claims.

Brief Description of the Drawings

Fig. 1(a) is a schematic diagram illustrating the structural organization of APP. The hatched box contains the sequence of the β/A_4 protein; the black box contains the so-called "Peptide 20" or couplone sequence; filled circles are N-glycosylation sites. The numbers designate amino acid sequence numbers corresponding to APP_{695}.

Fig. 1(b) is a bar graph illustrating the effects of synthetic APP peptides on G_o. In (b), (d), (e) and (f), values represent the mean ±S.E. of three experiments.

Fig. 1(c) is a graph illustrating the time course of the action of peptide 20 on G_o. Values represent the mean of three experiments. Since the S.E. was < 5% of each value in this figure, the error bars are not indicated.

Fig. 1(d) is a graph illustrating the effects of peptide 20 variants on G_o.

Fig. 1(e) is a graph illustrating the effect linkage with a transmembrane region has on the action of peptide 20 on G_o.

Fig. 1(f) is a graph illustrating the effect of pertussis toxin on peptide 20-induced stimulation of GTP-γS binding to G_o.

Figs. 2a-2d is a set of SDS-PAGE gels analyzed by immunoblotting, which illustrate the immunoprecipitation of APP and G_o by an anti-APP antibody from brain membranes. (a) Immunoprecipitation of APP by 22C11.

(b) Immunoprecipitation of G_o by 22C11. (c) Effect of Mg^{2+} on the immunoprecipitation of G_o by 22C11. (d) Effect of peptide 20 on 22C11-induced precipitation of $G_{o\alpha}$ (*left*) and APP (*right*). Each of the results presented in this figure was reproduced at least three times.

Fig. 3a is a schematic diagram of the construction method used to prepare recombinant mutant APP cDNAs. Regions labeled ATG, TAA, TGA signify original translation and termination sites and a newly inserted termination site, respectively.

Fig. 3b is a schematic diagram comparing the structures of authentic APP_{695} and the two recombinant mutant APP polypeptides, ΔN and ΔC.

Fig. 3c is an immunoblot analysis of Sf9 membranes using anti-Alz 90, 1C1, and 4G5.

Fig. 3d is an immunoblot analysis of the 22C11-precipitate from an Sf9 membrane-G_o reconstitution mixture.

Fig. 3e is an immunoblot illustrating dissociation of G_o from APP by activation of G_o. Each of the results presented in Figs. 3c-e was reproduced at least three times.

Fig. 4a is the cDNA sequence and deduced amino acid sequence of $G_{o1}\alpha$ (Strathmann et al., Proc. Natl. Acad. Sci. USA 87:6477-6481, 1990) (SEQ ID NO: 2).

Fig. 4b is the cDNA sequence and deduced amino acid sequence of $G_{o2}\alpha$ (Strathmann et al.) (SEQ ID NO: 28).

Detailed Description

It was previously shown that the insulin-like growth factor II receptor (IGF-IIR) couples directly to the G protein referred to as G_i (Nishimoto et al., J. Biol. Chem. 264:14029-14038, 1989) via a 14-residue section of the cytoplasmic tail of IGF-IIR, Arg^{2410}-Lys^{2423}

(Okamoto et al., Cell 62:709-717, 1990; Okamoto et al., Proc. Natl. Acad. Sci. U.S.A. 88:8020-8023, 1991). The structural determinants for the G_i-activating function in IGF-IIR were defined as (i) two basic residues at the N-terminal region of the amino acid sequence, and (ii) a C-terminal motif of *B-B-X-B* or *B-B-X-X-B* (where *B* is a basic residue and *X* is a non-basic residue) (Okamoto et al., Cell 62:709-717, 1990). To assess whether APP might function as a G protein-coupled receptor, the amino acid sequence of human APP695 was examined for regions of less than 26 residues which satisfy (i) and (ii). The sequence His^{657}-Lys^{676} is the only such region in the cytoplasmic domain of APP695. In two other isoforms of APP, APP751 (Ponte et al., Nature 331:525-527, 1988; Tanzi et al., Nature 331:528-530, 1988) and APP770 (Kitaguchi et al., Nature 331:530-532, 1988), as well as in mouse APP695 (Yamada et al., Biochem. Biophys. Res. Commun. 149:665-671, 1987), this sequence is completely conserved.

Preparation of peptides

A peptide corresponding to the His^{657}-Lys^{676} region of APP [HHGVVEVDAAVTPEERHLSK (SEQ ID NO: 1)] was synthesized and purified by standard methods using solid phase synthesis; this peptide is referred to as "peptide 20". Similarly prepared were peptides corresponding to other regions of APP_{695}:

APP(1-10), MLPGLALLLL (SEQ ID NO: 11);
APP(597-606), DAEFRHDSGY (SEQ ID NO: 12);
APP(677-695), MQQNGYENPTYKFFEQMQN (SEQ ID NO: 13);
and APP(639-648), TVIVITLVML (SEQ ID NO: 7), a portion of the transmembrane region of APP;

as well as the following variants of peptide 20:
HGVVEVDAAVTPEERHLSK (H-deleted, SEQ ID NO: 14);
GVVEVDAAVTPEERHLSK (HH-deleted, SEQ ID NO: 15);
HHGVVEVDAAVTPEE (RHLSK-deleted, SEQ ID NO: 16);

KQYTSIHHGVVEVDAAVTPEERHLSK (KQYTSI-added, SEQ ID NO: 17); and <u>TVIVITLVML</u>HHGVVEVDAAVTPEERHLSK (transmembrane region-connected peptide 20; SEQ ID NO: 18).

Peptides were purified by HPLC to greater than 95% purity, and were used immediately after synthesis.

Materials and Methods.

Trimeric G_o was purified to homogeneity from bovine brain as described (Katada et al., FEBS Lett. 213:353-358, 1987). This G_o preparation was stored in 20 mM Hepes/NaOH (pH 7.4), 1 mM EDTA, and 0.7% CHAPS, and diluted ≥ 10 fold for assays. $G_{i3\alpha}$, which was used in combination with 1.5-fold concentrated $G\beta\gamma$ (Okamoto et al., Natl. Acad. Sci. U.S.A. 88:8020-8023, 1991), was prepared as described by Morishita et al., Biochim. Biophys. Acta 161:1280-1285, 1989. Low molecular weight G proteins were prepared as described by Matsui et al., J. Biol. Chem. 263:11071-4, 1988; $G\beta\gamma$ was purified from bovine brain as set forth in Katada et al., FEBS Lett. 213:353-358, 1987.

GTPγS binding to G_o was assayed in a buffer containing 50 mM Hepes/NaOH (pH 7.4), 100 μM EDTA, 120 μM $MgCl_2$, and 60 nM [^{35}S]GTPγS (DuPont-New England Nuclear) at 37°C, and the fraction of total G_o bound to GTPγS was measured as described (Okamoto et al., Cell 62:709-717, 1990). GTPγS binding to peptides was negligible. The total amount of G_o in a given preparation was defined as the saturation amount of GTPγS bound to G_o following a 30-min incubation of G_o with 10 mM Mg^{2+} and ≥ 60 nM GTPγS at 30°C.

Reconstitution of G_o into phospholipid vesicles was accomplished with 1 mg/ml of phosphatidylcholine, using the gel filtration method (Nishimoto et al., J. Biol. Chem. 264:14029-14038, 1989). In a final

incubation for GTPγS binding, 5 nM of reconstituted G_o was used.

For experiments exploring the effect of Mg^{2+}, the Mg^{2+} concentration was set by using Mg-EDTA buffer (Birnbaumer et al., J. Eur. J. Biochem. 136:107-112, 1983).

Bovine brain membranes, prepared as described (Katada et al., FEBS Lett. 213:353-358, 1987) and suspended in buffer A [10 mM Hepes/NaOH (pH 7.4), 1 mM EDTA, 10 mM acetic acid, and 250 mM sucrose, plus a mixture (termed "PAL") of 2 mM PMSF, 20 μg/ml aprotinin, and 20 μM leupeptin], were centrifuged and the pellet was solubilized for 1 h at 4°C in buffer B (10 mM Hepes/NaOH (ph 7.4), 1 mM EDTA, 120 mM NaCl, 0.5% CHAPS, and PAL). Following centrifugation of the material at 15000 rpm for 1 h, the supernatant (500 μg protein, unless specified) was incubated in buffer C (20 mM Hepes/NaOH (pH 7.4), 1 mM EDTA, 120 mM NaCl, and PAL) and 2% BSA with 22C11-coated protein G-Sepharose, which had been prepared by incubating protein G-Sepharose (Pharmacia) with anti-APP monoclonal antibody 22C11 (Boehringer Mannheim) for 1 h at 4°C. An antibody concentration of ≥ 2 μg/ml was found to saturate precipitation of APP and G_o, so 2 μg/ml was the concentration used for immunoprecipitation studies. As a control, 2 μg/ml of rabbit IgG was used. After overnight shaking at 4°C, the immunoprecipitated sample was centrifuged at 5000 rpm for 5 min. The pellet was washed three times with ice-cold buffer C and the final pellet was applied to SDS-PAGE. Electroblotting onto a PVDF sheet was performed as described (Okamoto et al., J. Biol. Chem. 266:1085-1091, 1991). After blocking with PBS containing 2% skim milk and 1% BSA, the sheet was incubated with the first antibody [1 μg/ml of 22C11; 1/1000 dilution of anti-$G_o\alpha$ monoclonal antibody GC/2 (DuPont-New England Nuclear); 1/1000 dilution of 1C1, a

monoclonal antibody against the C-terminal peptide $_{677-695}$ of APP_{695}] for 4 h, and then exposed to horseradish peroxidase-conjugated goat IgG reactive for mouse or rabbit immunoglobulins for 2-4 h at room temperature. The antigenic bands were detected with an ECL detection kit (Amersham). YL1/2 (SERA Lab), an anti-tubulin antibody, was used at 1:500 dilution for immunodetection.

Effects of synthetic APP peptides on G proteins.

In the experiment shown in Fig. 1(b), 10 nM G_o was incubated with water or 100 μM of each peptide for 2 min, and the amount of GTPγS bound to G_o at the end of this period was measured. In the experiment shown in Fig. 1(c), 10nM G_o was incubated with water (○) or 100 μM peptide 20 (SEQ ID NO: 1) (●), and GTPγS binding was measured at the indicated times. From Fig. 1(d), it can be seen that peptide 20 (SEQ ID NO: 1) stimulated the rate constant of GTPγS binding to G_o in a dose-dependent manner, whereas Fig. 1(b) shows that peptides from other regions of APP695 were ineffective. GTPγS binding to G_o in the presence or absence of peptide 20 (SEQ ID NO: 1) obeyed first-order kinetics according to the equation

$$\ln[(B_T-B)/B_T]=-k_{app}t$$

(B is the binding at time t; B_T is the total binding observable at infinite time; and k_{app} is the rate constant for GTPγS binding). The ability of peptide 20 (SEQ ID NO: 1) to activate G_o was gradually decreased during storage at either -4°C or -20°C.

Studies using structural variant peptides suggest that both the N-terminal basic residues and the C-terminal *B-B-X-X-B* motif play essential roles in the G_o-activating function of peptide 20 (SEQ ID NO: 1) [Fig. 1(d)]. In this experiment, 10 nM G_o was incubated with various concentrations of HHGVVEVDAAVTPEERHLSK (peptide 20, SEQ ID NO: 1; □), HGVVEVDAAVTPEERHLSK (H-deleted, SEQ

ID NO: 14; ◊), GVVEVDAAVTPEERHLSK (HH-deleted, SEQ ID NO: 15; □), HHGVVEVDAAVTPEE (RHLSK-deleted, SEQ ID NO: 16; ♦), or KQYTSIHHGVVEVDAAVTPEERHLSK (KQYTSI-added, SEQ ID NO: 17; ■), and GTPγS binding to G_o at 2 min. was measured. Fig. 1(d) indicates which aspects of primary structure determine the G_o-activator function of peptide 20 (SEQ ID NO: 1). Deletion of either one or both of the N-terminal His residues nullified G_o-activator function of the peptide. The peptide (SEQ ID NO: 16) in which the C-terminal five residues of peptide 20 (SEQ ID NO: 1) has been deleted is several times less potent than peptide 20 (SEQ ID NO: 1).

As illustrated in Fig. 1(e), G_o reconstituted in phospholipid vesicles was incubated with transmembrane region-connected peptide 20 (<u>TVIVITLVML</u>HHGVVEVDAAVTPEERHLSK, SEQ ID NO: 18; □) or the partial sequence of the APP transmembrane domain alone (TVIVITLVML, SEQ ID NO: 7; □). Transmembrane region-connected peptide 20 (SEQ ID NO: 18) was also incubated with G_o in the absence of phospholipids and the presence of 0.07% CHAPS (♦). The transmembrane region-connected peptide 20 (SEQ ID NO: 18) stimulated G_o reconstituted in phospholipid vesicles with a potency 10 times greater than that of peptide 20 (SEQ ID NO: 1). The transmembrane region alone (SEQ ID NO: 7) was without effect on G_o. In the absence of phospholipids, transmembrane region-connected peptide 20 (SEQ ID NO: 18) showed an effect on G_o no more potent than peptide 20 (SEQ ID NO: 1). Therefore, the stimulatory action of this transmembrane region-connected peptide (SEQ ID NO: 18) is attributed to the peptide 20 (SEQ ID NO: 1) sequence; the potentiating effect of the transmembrane region may be exerted by interactions with phospholipids.

In the experiment shown in Fig. 1(f), ADP-ribosylation of G_o was accomplished by incubating G_o

reconstituted in phospholipid vesicles with 10 μg/ml preactivated pertussis toxin in the presence of 10μM NAD for 15 min at 30°C as described (Okamoto et al,, Cell 62:709-717, 1990). Preactivation of pertussis toxin (Funakoshi, Japan) was carried out by treating the toxin with 100 μM ATP and 1 mM DTT for 10 min at 30°C. Reconstitution of G_o into phospholipid vesicles was accomplished with 1 mg/ml phosphatidylcholine (Sigman, P-5638) at a final G_o concentration of 50.2 nM in a buffer containing 20 mM Hepes/NaOH (pH 7.4), 0.1 mM EDTA, 1 mM DTT, and 100 mM NaCl by the gel filtration method (Nishimoto et al., J. Biol. Chem. 264:14029-14038, 1989). In a final incubation for GTPγS binding, 5 nM of reconstituted G_o was used. Increasing concentrations of peptide 20 (SEQ ID NO: 1) were incubated for 2 min with G_o reconstituted in phospholipid vesicles which had been treated with pertussis toxin in the presence (♦) or absence (□) of NAD, and GTPγS binding to G_o was measured.

Although peptide 20 (SEQ ID NO: 1) produced 2-3 fold stimulation of GTPγS binding to G_o in the mid-range of Mg^{2+} concentrations, the effect of peptide 20 (SEQ ID NO: 1) could not be observed at low (≤ 100 nM) or high (≥ 10 mM) Mg^{2+} concentrations.

Peptide 20 (SEQ ID NO: 1) had little effect on G proteins other than G_o: G_{i1}, G_{i2}, G_{i3}, G_s, c-Ki-*ras* p21 and *smg* p25A were not stimulated by this peptide (data not shown). Thus, peptide 20 (SEQ ID NO: 1) activates G_o in a receptor-like manner, suggesting that APP interacts directly with G_o through the peptide 20 (SEQ ID NO: 1) region.

Coprecipitation of APP and G_o

In an effort to determine whether APP is linked to G_o in a native membrane environment, the coprecipitation studies shown in Fig. 2a were performed. Solubilized membranes of bovine brain were first immunoprecipitated

by monoclonal anti-APP antibody 22C11, and the immunoprecipitate was then probed by immunodetection with 22C11 (Lane 2) or 1C1, a monoclonal antibody against the C-terminal peptide$_{677-695}$ of APP (SEQ ID NO: 13; Lane 4). Lanes 1 and 3 of Fig. 2a indicate the controls in which either no solubilized membranes were included (Lane 1), or rabbit IgG was used for the precipitation step instead of antibody 22C11 (Lane 3). In each control, immunodetection was performed with 22C11. The 55-kDa and 25-kDa bands seen in Lanes 1 and 2 may be heavy and light chains of the 22C11 used for precipitation, which reacted with an anti-mouse IgG antibody during immunodetection. The precipitate by control rabbit IgG contained no detectable APP. Although the 100 kD molecular size of APP appears here to be slightly less than the 110-130 kD reported (Weidemann et al., Cell 57:115-126, 1989), the precipitated form is unlikely to be an extracellular fragment of APP, because 1C1 recognizes this 100-kDa band.

In the experiment illustrated in Fig. 2b, coprecipitation of various G proteins with APP was investigated. Bovine brain membrane preparations were immunoprecipitated with 22C11; the immunoprecipitated proteins were subjected to SDS-PAGE and immunoblotted with the indicated anti-G protein antisera (1/1000 dilution). Lane 2: GC/2, anti-$G_o\alpha$ antiserum; lane 3: GC/2 plus 1 μg/ml of purified G_o; lane 4: GA/1, common $G\alpha$ antiserum; lane 5: AS/7, anti-$Gi\alpha$ antiserum; lane 6: MS/1, common $G\beta$ antiserum. Lane 1 shows a control immunoblot with GC/2, in which a buffer solution rather than the bovine brain membrane preparation was immunoprecipitated with 22C11. Lane 7 indicates immunoblotting with GC/2 of the precipitate resulting from immunoprecipitation of brain membranes with control rabbit IgG, rather than 22C11. The identity of the 39-

kDa protein in lane 2 as G_o was verified by its absence in the non-membrane control (lane 1); by its staining with another $G_o\alpha$-specific antibody, αGO1 (Morishita et al., Eur. J. Biochem. 174:7-94, 1988) (data not shown); and by a diminution of staining of this band in the presence of excess soluble G_o (lane 3). The 22C11-precipitate also contained immunoreactivity of Gβ in a doublet at 35-36-kDa (lane 6). The 22C11-precipitate did not react with an anti-Giα antibody AS/7 (lane 5). The antibody GA/1 detected only a 39-kDa band in the 22C11-precipitate (lane 4). The control rabbit IgG immunoprecipitate did not produce anti-G_o-immunoreactive bands corresponding to either APP or G_o (lane 7). These experiments indicate that the 22C11-precipitate from brain membranes contains APP immunoreactivity at 100 kDa, $G_o\alpha$ immunoreactivity at 39 kDa, and Gβ immunoreactivity in a doublet at 35-36 kDa, but no detectable immunoreactivity indicating the presence of $G_i\alpha$ or other heterotrimeric G proteins. A tubulin antibody, YL1/2, did not stain the 22C11-precipitate (data not shown).

In the experiment shown in Fig. 2c, the effect of Mg^{2+} concentration on co-precipitation of G_o with anti-APP antibody was studied. 100 μg of solubilized brain membranes were precipitated by 22C11 in the presence of various Mg^{2+} concentrations controlled with Mg-EDTA buffer (Birnbaumer et al., J. Eur. J. Biochem. 136:107-112, 1983). The precipitates were analyzed by immunoblotting with GC/2. The control lane indicates the results of precipitation of brain membranes by rabbit IgG followed by immunodetection with GC/2. In the absence of Mg^{2+}, G_o was less efficiently co-precipitated by 22C11. Mg^{2+} concentrations between 1 μM and 1 mM resulted in maximal immunoprecipitation of G_o. At concentrations > 10 mM, relatively little G_o was precipitated. In contrast, immunoprecipitation of APP by 22C11 was not affected by

Mg^{2+} concentration (data not shown). These results indicate that, while Mg^{2+} is not absolutely required for complex formation by APP and G_o, the concentration of Mg^{2+} does strongly influence complex formation. A mid range of Mg^{2+} concentration was found to facilitate APP-G_o association.

Fig. 2d illustrates the results of an experiment indicating that peptide 20 (SEQ ID NO: 1) prevents the 22C11-mediated co-precipitation of G_o, whereas it did not affect the precipitation of APP by 22C11. In contrast, a control peptide (SEQ ID NO: 13) representing a segment of APP different from that represented by peptide 20 (SEQ ID NO: 1) had no discernable effect on 22C11-mediated co-precipitation of G_o. In this experiment, solubilized brain membranes were incubated with 22C11-coated beads in the presence of 10 μM peptide 20 (SEQ ID NO: 1; 2nd and 5th lanes) or 10 μM of the control peptide, peptide$_{677-695}$ of APP (SEQ ID NO: 13; 3rd and 6th lanes), or in the absence of both of these peptides (1st and 4th lanes). In this experiment, an anti-mouse IgG antibody different from that used in (a) was employed.

Precipitation of G_o reconstituted with recombinant APP-antibody complex

A baculovirus DNA encoding full-length APP_{695} (SEQ ID NO: 9) was prepared as outlined in Fig. 3a. Authentic mouse APP_{695} cDNA (SEQ ID NO: 9) was provided by Dr. Yoshiyuki Sakaki (University of Tokyo, Japan) (Yamada et al., Biochem. Biophys. Res. Commun. 149:665-671, 1987) in the vector pUC18. The HindIII-BamHI fragment containing the entire coding region was initially subcloned into the vector pBR322 (pBR-APP). A single BamHI site was inserted immediately before the ATG codon of the HindIII-SphI fragment. This BamHI site was inserted to permit efficient expression of the encoded APP protein in

baculovirus-infected cells. The BamHI site-inserted APP_{695}-coding DNA (BamHI-APP_{695}) was constructed from the HindIII-SphI fragment and pBR-APP, utilizing their internal KpnI sites, and subcloned into pUC18. By using BamHI-APP_{695} as template, two truncation mutants were generated and subcloned into pUC18. These mutants possess an insertion of two TGA codons immediately before (ΔN) or after (ΔC) the peptide 20 sequence. Each BamHI-BamHI fragment of these respective APP-variation-encoding pUC18 plasmids was inserted into the baculovirus transfer/expression vector pVL1393 (Invitrogen). The entire region that had been through a single-stranded intermediate was sequenced to confirm the absence of unwanted nucleotide changes. New insertions were generated by oligonucleotide-directed mutagenesis with a kit (Takara) by the method of Kunkel et al. (Meth. Enzymol. 154:367-382, 1987). For the insertion of a BamHI site, a restriction fragment encoding the ATG start codon was subcloned into the vector M13mp18 and a single stranded template was generated. An oligonucleotide primer (CCACGCAGGATCACGGGATCCATGCTGCCCAGCTTG; SEQ ID NO: 19) was used to introduce GGATCC (SEQ ID NO: 20) immediately before the start codon. Following primer extension, the phage was used to transform E. coli strain JM109. Plaques were selected and single stranded DNA was sequenced. A restriction fragment containing the mutated region was subcloned into pBR-APP. For the insertion of the stop codons, oligonucleotide primers [CAGTACACATCCATCTGATGACATCATGGCGTGGTG (SEQ ID NO: 21) and CGCCATCTCTCCAGTGATGAATGCAGCAGAACGGA (SEQ ID NO: 22)] and the M13mp19 vector were used to introduce two sequential TGA stop codons. Using the method of Summers and Smith (Summers et al., Tex. Agric. Exp. Stn. Bull. 1555, 1987), baculoviruses incorporating these APP cDNAs were generated using selection by immunoblot analysis with

22C11, and recovered by infecting Sf9 cells (Invitrogen). Four days after treatment of Sf9 cells with the viruses, cells were homogenized and suspended in buffer A. After the solubilization of the pellet with buffer B, the supernatant (100 μg) was mixed overnight with 22C11-coated protein G-Sepharose in buffer C plus 2% BSA at 4°C on a shaker. After centrifugation, the precipitated beads were incubated with purified G_o (1 μg) in buffer C supplemented with 1.1 mM MgCl2 and 2% BSA for 8-24 h at 4°C on a shaker. After washing four times with ice-cold buffer C, the centrifugation precipitate was subjected to SDS-PAGE, electroblotting, and immunodetection with the first antibodies (1 μg/ml of 22C11; 10 μg/ml of anti-Alz 90; 1/1000 dilution of 1C1; 1/500 dilution of 4G5; 0.1 μg/ml of αGO1) and the second goat anti-mouse or anti-rabbit IgGs conjugated with HRP. (Immunodetection of 1C1 and 4G5, both of which are mouse IgM (κ), was accomplished using as second antibody a mixture of HRP-conjugated anti-rabbit IgG, rabbit anti-mouse IgM and rabbit anti-mouse κ antibodies.) The three APP constructs prepared as described above are compared in the schematic diagram of Fig. 3b. The polypeptides encoded by all three constructs retain the entire transmembrane and extracellular domains of APP; while ΔN (SEQ ID NO: 23) lacks all of the peptide 20 residues as well as the sequence on the carboxy terminal side of the peptide 20 region, ΔC (SEQ ID NO: 24) retains the peptide 20 sequence and is missing only the latter sequence.

Sf9 cells were infected, using standard methods, by recombinant baculoviruses encoding full length APP_{695} cDNA (SEQ ID NO: 9), APP_{1-656} cDNA (ΔN; SEQ ID NO: 23), or APP_{1-676} cDNA (ΔC; SEQ ID NO: 24). In uninfected Sf9 cells, no immunoreactivity for anti-APP or anti-G_o antibodies was detected (data not shown). The membranes

of Sf9 cells infected with the baculoviruses encoding APP_{695} (SEQ ID NO: 9), ΔN (SEQ ID NO: 23), and ΔC (SEQ ID NO: 24) genes (referred to as Sf9-APP_{695}, Sf9-ΔN, and Sf9-ΔC, respectively) were found to express, respectively, 130-, 120- and 130-kDa proteins reactive with antibody 22C11 (Fig. 3d, right side). The Sf9-APP_{695} cells expressed APP at ≈ 0.1% of the total membrane protein. When the membranes of the three types of infected cells were immunoprecipitated with antibody Anti-Alz 90 (Boehringer Mannheim), a mouse monoclonal antibody specific for an epitope corresponding to to residues 551-608 of APP (SEQ ID NO: 25; a section of APP that is within the extracellular domain), 130-kDa, 120-kDa, and 130-kDa proteins were recognized in Sf9-APP_{695}, Sf9-ΔN, and Sf9-ΔC cells, respectively (Fig. 3c, top panel). Membranes from all three types of infected cells showed approximately equivalent reactivity to the antibody, indicating that at least this portion of the extracellular domain was intact on each of the three and that all three cell types express approximately equal amounts of recombinant protein. When the antibody used was 1C1, a mouse monoclonal prepared against a peptide corresponding to residues 677-695 of APP (SEQ ID NO: 13), only Sf9-APP_{695} membranes were reactive, indicating that the region corresponding to the C-terminal portion of the cytoplasmic domain is missing from both ΔN (SEQ ID NO: 23) and ΔC (SEQ ID NO: 24) (Fig. 3c, middle panel). When the antibody used was 4G5, a mouse monoclonal antibody raised against a peptide corresponding to residues 657-676 of APP (SEQ ID NO: 1; the peptide 20 region of the cytoplasmic domain), 130 kDa bands from both Sf9-APP_{695} and Sf9-ΔC membranes reacted with the antibody, but Sf9-ΔN membranes did not, a demonstration that ΔN (SEQ ID NO: 23) but not ΔC (SEQ ID NO: 24) lacks the peptide 20 region of APP (Fig. 3c, bottom panel).

These experiments clearly indicate that the expressed proteins are recombinant APP_{1-695} (SEQ ID NO: 9), APP_{1-656} (SEQ ID NO: 23), and APP_{1-676} (SEQ ID NO: 24), respectively, as designed.

The 22C11-precipitates from these Sf9 membranes expressing various forms of APP were exposed to purified G_o, reprecipitated with 22C11, and subjected to immunoblot analysis using anti-$G_o\alpha$ antibody αGO1 (Fig. 3d, left four lanes) and by 22C11 (right four lanes). αGO1 (Morishita et al., Eur. J. Biochem. 174:87-94, 1988) was provided by Dr. Tomiko Asano; similar results were obtained when antibody GC/2 was substituted. The control lanes are 22C11-precipitate exposed to G_o in the absence of Sf9 membranes. Approximately 1/10-1/20 (0.05-0.1 μg/tube) of the reconstituted G_o was precipitated, together with a comparable amount ($\approx$0.1 μg/tube) of APP. Easily detectable amounts of $G_o\alpha$ were present in the final precipitate when G_o was mixed with 22C11-precipitates from Sf9-ΔC or Sf9-APP695 membranes, but essentially no $G_o\alpha$ was found in the final precipitate from Sf9-ΔN membranes. Thus, formation of an APP-G_o complex requires the peptide 20 region, residues 657-676 (SEQ ID NO: 1).

In the experiment illustrated in Fig. 3e, 22C11-precipitates from Sf9-APP_{695} membranes (100 μg protein each) were incubated with activated G_o (lanes 2 and 4) or unactivated G_o (lanes 1 and 3); the final precipitates (left panel) and supernatants (right panel) were analyzed by simultaneous immunoblotting with 22C11 and αGO1 antibodies. Activation of G_o was carried out by incubating G_o in 20 mM Hepes/NaOH (pH 7.4), 1 mM EDTA, 2 mM $MgCl_2$, and 1 μM GTPγS overnight at room temperature. When G_o was incubated with GTPγS, no $G_o\alpha$ associated with the APP-22C11 complex (Fig. 3e), suggesting that the

activation state of the G protein regulates APP-G_o association.

This study suggests that APP functions as a receptor coupled to G_o through the G_o-activator cytoplasmic domain His^{657}-Lys^{676} (SEQ ID NO: 1). APP has a point mutation in at least one form of familial Alzheimer's disease (Goate et al., Nature 349:704-706, 1991). A structural alteration of APP is therefore thought to be one cause of Alzheimer's disease, although it remains unknown how the mutation might produce the disease. One novel possibility suggested by this study is that the cytoplasmic, C-terminal fragment of APP is pathogenic. It has been suggested (Abraham et al., Biotechnology 7:147-153, 1989; Shivers et al., EMBO J. 7:1365-1370, 1988; Kametani et al., Biomedical Research 10:179-183, 1989) that the residual C-terminal portion of APP may remain in the cell membrane after abnormal cleavage of APP to produce β/A4 protein in Alzheimer's disease neurons. By analogy with the oncogenic transformation of c-*erb* B into v-*erb* B, such a structural alteration of APP may alter its function and prompt APP to constitutively activate G_o. This hypothesis is consistent with the study (Yanker et al., Science 245:417-420, 1989) indicating that recombinant expression of the C-terminal 105-residue portion of APP in neuronal cells evokes cell death, and with the reports that G_o activity is linked to neuronal growth cone motility (Strittmatter et al., BioEssays 13:127-134, 1990), axon and dendrite formation (Granneman et al., J. Neurochemistry 54:1995-2001, 1990), and memory (Guillen et al., EMBO J. 9:1449-1455, 1990). This study suggests that Alzheimer's disease is a disorder of an APP-G_o signalling system caused by structural alterations of APP.

Example 1

The screening method of the invention can be carried out as follows:

The assay used can be a very simple cell-free assay employing a first polypeptide consisting essentially of the couplone, or G_o-binding portion, of APP (SEQ ID NO: 1) and a second polypeptide consisting essentially of an APP-binding portion of G_o. This APP-binding portion of G_o may be the 15-residue segment identified as the anticouplone portion of G_o (SEQ ID NO: 3), or it may be one or both of the two flanking regions, residues 1-3 (SEQ ID NO: 4) and residues 19-36 (SEQ ID NO: 5) of G_o. Alternatively, longer portions, or all, of APP and/or G_o can be used, or the appropriate portions of APP and/or G_o can be linked to other polypeptides to form hybrid polypeptides with characteristics (such as altered immunoreactivity or enzymatic activity) that would improve detection of the endpoint of the assay. The assay is carried out by contacting the APP-based polypeptide with the G_o-based polypeptide in the presence of a candidate compound, in parallel with a control assay containing no candidate compound, and determining whether the candidate compound inhibits co-immunoprecipitation of the first and second polypeptides (using either an antibody specific for the first polypeptide or an antibody specific for the second polypeptide). Alternatively, activation of the second (G_o) polypeptide may be the measured criterion: if so, the second polypeptide must include the GTP-binding region of G_o (SEQ ID NO: 10), and GTP or an appropriate non-hydrolyzable analog thereof (such as GTPγS or Gpp(NH)p) must be included in the assay. The assay may also be carried out using phospholipid vesicles prepared by standard methods (e.g., as described by Nishimoto et al., J. Biol. Chem. 264:14029-14038, 1989), provided that

the first (APP) polypeptide includes a region of hydrophobic amino acids [such as all (SEQ ID NO: 8) or a portion (e.g., SEQ ID NO: 7) of the transmembrane region of APP) that permit it to be anchored in the phospholipid bilayer. Alternatively, the assay may be carried out using intact cells or red cell ghosts which contain APP and G_o, or appropriate portions thereof. The cells may express the first and second polypeptides naturally or by virtue of genetic engineering, or the polypeptides may be introduced directly into the cells or ghosts by standard means.

Example 2

The progress of Alzheimer's disease may be halted or reversed by treating a patient with a compound which diminishes the activation of neural G_o by truncated APP. Such a compound may be identified in a screening assay as described above, or may consist essentially of a polypeptide containing the amino acid sequence of (a) the couplone region of APP (SEQ ID NO: 1), (b) the anticouplone region of G_o (SEQ ID NO: 3), or (c) the APP-associating region(s) of G_o (SEQ ID NO: 4 and/or 5), or a combination of (b) and (c). Such polypeptides may be produced in quantity by standard recombinant means, or by standard synthetic techniques. To minimize proteolytic degradation *in vivo*, the carboxy and amino termini may be derivatized (e.g., with ester or amide groups), some or all of the amino acids may be replaced with D-amino acids, or particularly sensitive peptide linkages may be substituted with non-peptide bonds using standard methodology. To improve penetration of the blood-brain barrier (BBB), the polypeptides may be altered to increase lipophilicity (e.g., by esterification to a bulky lipophilic moiety such as cholesteryl) or to supply a cleavable "targetor" moiety that enhances retention on

the brain side of the barrier (Bodor et al., Science 257:1698-1700, 1992). Alternatively, the polypeptide may be linked to an antibody to the transferrin receptor, in order to exploit that receptor's role in transporting iron across the blood-brain barrier, as taught by Friden et al., Science 259:373-377, 1993. It is expected that an intravenous dosage equivalent to approximately 1 to 100 μmoles of the polypeptide of the invention per kg per day, or an intrathecally administered dosage of approximately 0.1 to 50 μmoles per kg per day, will be effective in blocking activation of G_o in an Alzheimer's patient. If the polypeptide is sufficiently protected from proteolytic degradation, as described above, it may also be administered orally in appropriately higher doses. Alternatively, the compound may be incorporated into a slow-release implant to ensure a relatively constant supply of the therapeutic to the patient's brain.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Nishimoto, Ikuo

(ii) TITLE OF INVENTION: ALZHEIMER'S DISEASE THERAPEUTICS

(iii) NUMBER OF SEQUENCES: 30

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(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb
(B) COMPUTER: IBM PS/2 Model 50Z or 55SX
(C) OPERATING SYSTEM: MS-DOS (Version 5.0)
(D) SOFTWARE: WordPerfect (Version 5.1)

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: 08/019,208
(B) FILING DATE: February 18, 1993
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:

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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
 1               5                  10                  15

His Leu Ser Lys
            20
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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1910
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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TGTGGCAGGG AAGGGGCCAC C ATG GGA TGT ACG CTG AGC GCA GAG GAG AGA        51
                        Met Gly Cys Thr Leu Ser Ala Glu Glu Arg
                         1               5                  10

GCC GCC CTC GAG CGG AGC AAG GCG ATT GAG AAA AAC CTA AAA GAA GAT        99
Ala Ala Leu Glu Arg Ser Lys Ala Ile Glu Lys Asn Leu Lys Glu Asp
                15                  20                  25

GGC ATC AGC GCC GCC AAA GAC GTG AAA TTA CTC CTG CTG GGG GCT GGA       147
Gly Ile Ser Ala Ala Lys Asp Val Lys Leu Leu Leu Leu Gly Ala Gly
            30                  35                  40

GAA TCA GGA AAA AGC ACC ATT GTG AAG CAG ATG AAG ATC ATC CAT GAA       195
Glu Ser Gly Lys Ser Thr Ile Val Lys Gln Met Lys Ile Ile His Glu
        45                  50                  55

GAT GGC TTC TCT GGG GAA GAC GTG AAG CAG TAC AAG CCT GTG GTC TAC       243
Asp Gly Phe Ser Gly Glu Asp Val Lys Gln Tyr Lys Pro Val Val Tyr
    60                  65                  70

AGC AAC ACC ATC CAG TCT CTG GCG GCC ATT GTC CGG GCC ATG GAC ACT       291
Ser Asn Thr Ile Gln Ser Leu Ala Ala Ile Val Arg Ala Met Asp Thr
75                  80                  85                  90

TTG GGC GTG GAG TAT GGT GAC AAG GAG AGG AAG ACG GAC TCC AAG ATG       339
Leu Gly Val Glu Tyr Gly Asp Lys Glu Arg Lys Thr Asp Ser Lys Met
                95                 100                 105

GTG TGT GAC GTG GTG AGT CGT ATG GAA GAC ACT GAA CCG TTC TCT GCA       387
Val Cys Asp Val Val Ser Arg Met Glu Asp Thr Glu Pro Phe Ser Ala
            110                 115                 120

GAA CTT CTT TCT GCC ATG ATG CGA CTC TGG GGC GAC TCG GGG ATC CAG       435
Glu Leu Leu Ser Ala Met Met Arg Leu Trp Gly Asp Ser Gly Ile Gln
        125                 130                 135

GAG TGC TTC AAC CGA TCT CGG GAG TAT CAG CTC AAT GAC TCT GCC AAA       483
Glu Cys Phe Asn Arg Ser Arg Glu Tyr Gln Leu Asn Asp Ser Ala Lys
    140                 145                 150

TAC TAC CTG GAC AGC CTG GAT CGG ATT GGA GCC GGT GAC TAC CAG CCC       531
Tyr Tyr Leu Asp Ser Leu Asp Arg Ile Gly Ala Gly Asp Tyr Gln Pro
155                 160                 165                 170
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ACT GAG CAG GAC ATC CTC CGA ACC AGA GTC AAA ACA ACT GGC ATC GTA        579
Thr Glu Gln Asp Ile Leu Arg Thr Arg Val Lys Thr Thr Gly Ile Val
                175                 180                 185

GAA ACC CAC TTC ACC TTC AAG AAC CTC CAC TTC AGG CTG TTT GAC GTC        627
Glu Thr His Phe Thr Phe Lys Asn Leu His Phe Arg Leu Phe Asp Val
            190                 195                 200

GGG GGC CAG CGA TCT GAA CGC AAG AAG TGG ATC CAC TGC TTT GAG GAT        675
Gly Gly Gln Arg Ser Glu Arg Lys Lys Trp Ile His Cys Phe Glu Asp
        205                 210                 215

GTC ACG GCC ATC ATC TTC TGT GTC GCA CTC AGC GGC TAT GAC CAG GTG        723
Val Thr Ala Ile Ile Phe Cys Val Ala Leu Ser Gly Tyr Asp Gln Val
    220                 225                 230

CTC CAC GAG GAC GAA ACC ACG AAC CGC ATG CAC GAG TCT CTC ATG CTC        771
Leu His Glu Asp Glu Thr Thr Asn Arg Met His Glu Ser Leu Met Leu
235                 240                 245                 250

TTC GAC TCC ATC TGT AAC AAC AAG TTT TTC ATT GAT ACC TCC ATC ATC        819
Phe Asp Ser Ile Cys Asn Asn Lys Phe Phe Ile Asp Thr Ser Ile Ile
                255                 260                 265

CTC TTC CTC AAC AAG AAA GAC CTC TTT GGC GAG AAG ATT AAG AAG TCA        867
Leu Phe Leu Asn Lys Lys Asp Leu Phe Gly Glu Lys Ile Lys Lys Ser
            270                 275                 280

CCC TTG ACC ATC TGC TTT CCC GAA TAC CCA GGC TCC AAC ACC TAT GAA        915
Pro Leu Thr Ile Cys Phe Pro Glu Tyr Pro Gly Ser Asn Thr Tyr Glu
        285                 290                 295

GAT GCA GCT GCC TAC ATC CAA ACA CAG TTT GAA AGC AAA AAC CGC TCA        963
Asp Ala Ala Ala Tyr Ile Gln Thr Gln Phe Glu Ser Lys Asn Arg Ser
    300                 305                 310

CCC AAC AAA GAA ATT TAC TGT CAC ATG ACT TGT GCC ACA GAC ACG AAT       1011
Pro Asn Lys Glu Ile Tyr Cys His Met Thr Cys Ala Thr Asp Thr Asn
315                 320                 325                 330

AAT ATC CAG GTG GTA TTC GAC GCC GTC ACC GAC ATC ATC ATT GCC AAC       1059
Asn Ile Gln Val Val Phe Asp Ala Val Thr Asp Ile Ile Ile Ala Asn
                335                 340                 345

AAT CTC CGG GGC TGC GGC TTG TAC TGACCTCTTG TCCTGTATAG CAACCTATTT      1113
Asn Leu Arg Gly Cys Gly Leu Tyr
            350

GACTGCTTCA TGGACTCTTT GCTGTTGATG TTGATCTCCT GGTAGCATGA CCTTTGGCCT     1173

TTGTAAGACA CACAGCCTTT CTGTACCAAG CCCCTGTCTA ACCTACGACC CCAGAGTGAC     1233

TGACGGCTGT GTATTTCTGT AGAATGCTGT AGAATACAGT TTTAGTTGAG TCTTTACATT     1293

TAGAACTTGA AAGGATTTTA AAAAACAAAA CAAAAACCAT TTCTCATGTG CTTTGTAGCT     1353

TTAAAAGAAA AAAGGAAAAC TCACCATTTA ATCCATATTT CCTTTTTATT TTGAAGTTTA     1413

AAAAAAAAAT GTCTGTACCC ACACCCTCCC CCTTCCCCAC CTCAGCAGAA CTGGGGCTGG     1473

CACACAGAGG CAGTGCTGGG CCTGGCGCCT CCCAGGGCTT CTGTGCAGCC CATGGCTGGT     1533

GGGAACATGT CAGGCTAGTC TGTCTAGAAG GCCACTGGCC ACTGTACCCA CCCTTCCCCA     1593
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TGCCTGTGGG CTGCCCAGAC ACCTCATATA CCACCAGGCA GTGGCAGCTC CGCCCTGCTC 1653

AGCCATGCGA CTCCAAACAC ACTCAAAGTT TGCGTAGAAA AAGCACAGCT CTGGCAGGGG 1713

TAGCTGCCAC AGACAACGCT CATCACCTAT AGAAATCCAG CCCTATAGAA GCAATTCACC 1773

CAGCCCCTTC CTACACTCCC TTTGTGTTGT TAACTTTTTG GTTTTTCTGG TCCTAGTGAG 1833

TGCCTCCCAT GCATACCTGA CCAGCTCTGC CAGTGTCTGG GGTCTGGGGA ACAGGGGTTG 1893

TGTGGTTTGG TTTTTGG 1910

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Asp Ala Val Thr Asp Ile Ile Ile Ala Lys Asn Leu Arg Gly Cys
1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Gly Cys
1

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Ile Glu Lys Asn Leu Lys Glu Asp Gly Ile Ser Ala Ala Lys Asp Val
1 5 10 15

Lys Leu

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 47
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

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Lys Lys Lys Gln Tyr Thr Ser Ile His His Gly Val Val Glu Val Asp
 1               5                  10                  15

Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys Met Gln Gln Asn
            20                  25                  30

Gly Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln Met Gln Asn
        35                  40                  45
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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

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Thr Val Ile Val Ile Thr Leu Val Met Leu
 1               5                  10
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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

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Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
 1               5                  10                  15

Ile Val Ile Thr Leu Val Met Leu
            20
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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2085
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

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ATG CTG CCC GGT TTG GCA CTG CTC CTG CTG GCC GCC TGG ACG GCT CGG      48
Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg
1               5                   10                  15

GCG CTG GAG GTA CCC ACT GAT GGT AAT GCT GGC CTG CTG GCT GAA CCC      96
Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
            20                  25                  30

CAG ATT GCC ATG TTC TGT GGC AGA CTG AAC ATG CAC ATG AAT GTC CAG     144
Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
        35                  40                  45

AAT GGG AAG TGG GAT TCA GAT CCA TCA GGG ACC AAA ACC TGC ATT GAT     192
Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
    50                  55                  60

ACC AAG GAA GGC ATC CTG CAG TAT TGC CAA GAA GTC TAC CCT GGA CTG     240
Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Gly Leu
65                  70                  75                  80

CAG ATC ACC AAT GTG GTA GAA GCC AAC CAA CCA GTG ACC ATC CAG AAC     288
Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
                85                  90                  95

TGG TGC AAG CGG GGC CGC AAG CAG TGC AAG ACC CAT CCC CAC TTT GTG     336
Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
            100                 105                 110

ATT CCC TAC CGC TGC TTA GTT GGT GAG TTT GTA AGT GAT GCC CTT CTC     384
Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
        115                 120                 125

GTT CCT GAC AAG TGC AAA TTC TTA CAC CAG GAG AGG ATG GAT GTT TGC     432
Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
    130                 135                 140

GAA ACT CAT CTT CAC TGG CAC ACC GTC GCC AAA GAG ACA TGC AGT GAG     480
Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145                 150                 155                 160

AAG AGT ACC AAC TTG CAT GAC TAC GGC ATG TTG CTG CCC TGC GGA ATT     528
Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
                165                 170                 175

GAC AAG TTC CGA GGG GTA GAG TTT GTG TGT TGC CCA CTG GCT GAA GAA     576
Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
            180                 185                 190

AGT GAC AAT GTG GAT TCT GCT GAT GCG GAG GAG GAT GAC TGC GAT GTC     624
Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Cys Asp Val
        195                 200                 205

TGG TGG GGC GGA GCA GAC ACA GAC TAT GCA GAT GGG AGT GAA GAC AAA     672
Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
    210                 215                 220

GTA GTA GAA GTA GCA GAG GAG GAA GAA GTG GCT GAG GTG GAA GAA GAA     720
Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225                 230                 235                 240
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GAA GCC GAT GAT GAC GAG GAC GAT GAG GAT GGT GAT GAG GTA GAG GAA 768
Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
245 250 255

GAG GCT GAG GAA CCC TAC GAA GAA GCC ACA GAG AGA ACC ACC AGC ATT 816
Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
260 265 270

GCC ACC ACC ACC ACC ACC ACC ACA GAG TCT GTG GAA GAG GTG GTT CGA 864
Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
275 280 285

GTT CCT ACA ACA GCA GCC AGT ACC CCT GAT GCC GTT GAC AAG TAT CTC 912
Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
290 295 300

GAG ACA CCT GGG GAT GAG AAT GAA CAT GCC CAT TTC CAG AAA GCC AAA 960
Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
305 310 315 320

GAG AGG CTT GAG GCC AAG CAC CGA GAG AGA ATG TCC CAG GTC ATG AGA 1008
Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
325 330 335

GAA TGG GAA GAG GCA GAA CGT CAA GCA AAG AAC TTG CCT AAA GCT GAT 1056
Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
340 345 350

AAG AAG GCA GTT ATC CAG CAT TTC CAG GAG AAA GTG GAA TCT TTG GAA 1104
Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
355 360 365

CAG GAA GCA GCC AAC GAG AGA CAG CAG CTG GTG GAG ACA CAC ATG GCC 1152
Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
370 375 380

AGA GTG GAA GCC ATG CTC AAT GAC CGC CGC CGC CTG GCC CTG GAG AAC 1200
Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
385 390 395 400

TAC ATC ACC GCT CTG CAG GCT GTT CCT CCT CGG CCT CGT CAC GTG TTC 1248
Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
405 410 415

AAT ATG CTA AAG AAG TAT GTC CGC GCA GAA CAG AAG GAC AGA CAG CAC 1296
Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
420 425 430

ACC CTG AAG CAT TTC GAG CAT GTG CGC ATG GTG GAT CCC AAG AAA GCC 1344
Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
435 440 445

GCT CAG ATC CGG TCC CAG GTT ATG ACA CAC CTC CGT GTG ATT TAT GAG 1392
Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
450 455 460

CGC ATG AAT CAG TCT CTC TCC CTG CTC TAC AAC GTG CCT GCA GTG GCC 1440
Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala
465 470 475 480

GAG GAG ATT CAG GAT GAA GTT GAT GAG CTG CTT CAG AAA GAG CAA AAC 1488
Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn
485 490 495

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TAT TCA GAT GAC GTC TTG GCC AAC ATG ATT AGT GAA CCA AGG ATC AGT      1536
Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser
            500                 505                 510

TAC GGA AAC GAT GCT CTC ATG CCA TCT TTG ACC GAA ACG AAA ACC ACC      1584
Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr
        515                 520                 525

GTG GAG CTC CTT CCC GTG AAT GGA GAG TTC AGC CTG GAC GAT CTC CAG      1632
Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln
    530                 535                 540

CCG TGG CAT TCT TTT GGG GCT GAC TCT GTG CCA GCC AAC ACA GAA AAC      1680
Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn
545                 550                 555                 560

GAA GTT GAG CCT GTT GAT GCC CGC CCT GCT GCC GAC CGA GGA CTG ACC      1728
Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr
                565                 570                 575

ACT CGA CCA GGT TCT GGG TTG ACA AAT ATC AAG ACG GAG GAG ATC TCT      1776
Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
            580                 585                 590

GAA GTG AAG ATG GAT GCA GAA TTC CGA CAT GAC TCA GGA TAT GAA GTT      1824
Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
        595                 600                 605

CAT CAT CAA AAA TTG GTG TTC TTT GCA GAA GAT GTG GGT TCA AAC AAA      1872
His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
    610                 615                 620

GGT GCA ATC ATT GGA CTC ATG GTG GGC GGT GTT GTC ATA GCG ACA GTG      1920
Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
625                 630                 635                 640

ATC GTC ATC ACC TTG GTG ATG CTG AAG AAG AAA CAG TAC ACA TCC ATT      1968
Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
                645                 650                 655

CAT CAT GGT GTG GTG GAG GTT GAC GCC GCT GTC ACC CCA GAG GAG CGC      2016
His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
            660                 665                 670

CAC CTG TCC AAG ATG CAG CAG AAC GGC TAC GAA AAT CCA ACC TAC AAG      2064
His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
        675                 680                 685

TTC TTT GAG CAG ATG CAG AAC                                          2085
Phe Phe Glu Gln Met Gln Asn
    690                 695
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Lys Leu Leu Leu Leu Gly Ala Gly Glu Ser Gly Lys Ser Thr Ile Val
1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Leu Pro Gly Leu Ala Leu Leu Leu Leu
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr
1 5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln
1 5 10 15

Met Gln Asn

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg His
1 5 10 15

Leu Ser Lys

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg His Leu
1 5 10 15

Ser Lys

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu
1 5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Lys Gln Tyr Thr Ser Ile His His Gly Val Val Glu Val Asp Ala Ala
1 5 10 15

Val Thr Pro Glu Glu Arg His Leu Ser Lys
20 25

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

```
Thr Val Ile Val Ile Thr Leu Val Met Leu His His Gly Val Val Glu
  1               5                  10                  15

Val Asp Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys
             20                  25                  30
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

CCACGCAGGA TCACGGGATC CATGCTGCCC AGCTTG 36

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

GGATCC 6

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CAGTACACAT CCATCTGATG ACATCATGGC GTGGTG 36

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

CGCCATCTCT CCAGTGATGA ATGCAGCAGA ACGGA 35

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 656
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

```
Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg
1               5                   10                  15

Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
            20                  25                  30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
        35                  40                  45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
    50                  55                  60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Gly Leu
65                  70                  75                  80

Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
                85                  90                  95

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
            100                 105                 110

Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
        115                 120                 125

Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
    130                 135                 140

Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145                 150                 155                 160

Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
                165                 170                 175

Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
            180                 185                 190

Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Cys Asp Val
        195                 200                 205
```

Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
210 215 220

Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225 230 235 240

Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
245 250 255

Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
260 265 270

Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
275 280 285

Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
290 295 300

Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
305 310 315 320

Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
325 330 335

Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
340 345 350

Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
355 360 365

Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
370 375 380

Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
385 390 395 400

Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
405 410 415

Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
420 425 430

Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
435 440 445

Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
450 455 460

Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala
465 470 475 480

Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn
485 490 495

Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser
500 505 510

Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr
515 520 525

Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln
530 535 540

Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn
545 550 555 560

Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr
565 570 575

Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
580 585 590

Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
595 600 605

His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
610 615 620

Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
625 630 635 640

Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
645 650 655

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 676
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg
1 5 10 15

Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
20 25 30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
35 40 45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
50 55 60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Gly Leu
65 70 75 80

Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
85 90 95

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
100 105 110

Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
115 120 125

Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
130 135 140

Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145 150 155 160

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Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
                165                 170                 175

Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
            180                 185                 190

Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Cys Asp Val
        195                 200                 205

Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
    210                 215                 220

Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225                 230                 235                 240

Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
                245                 250                 255

Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
            260                 265                 270

Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
        275                 280                 285

Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
    290                 295                 300

Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
305                 310                 315                 320

Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
                325                 330                 335

Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
            340                 345                 350

Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
        355                 360                 365

Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
    370                 375                 380

Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
385                 390                 395                 400

Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
                405                 410                 415

Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
            420                 425                 430

Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
        435                 440                 445

Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
    450                 455                 460

Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala
465                 470                 475                 480

Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn
                485                 490                 495
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Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser
500 505 510

Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr
515 520 525

Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln
530 535 540

Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn
545 550 555 560

Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr
565 570 575

Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
580 585 590

Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
595 600 605

His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
610 615 620

Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
625 630 635 640

Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
645 650 655

His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
660 665 670

His Leu Ser Lys
675

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 58
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Ala Asp Ser Val Pro Ala Asn Thr Glu Asn Glu Val Glu Pro Val Asp
1 5 10 15

Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr Thr Arg Pro Gly Ser Gly
20 25 30

Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser Glu Val Lys Met Asp Ala
35 40 45

Glu Phe Arg His Asp Ser Gly Tyr Glu Val
50 55

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 56
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Val Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser
1 5 10 15

Ile His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu
20 25 30

Arg His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr
35 40 45

Lys Phe Phe Glu Gln Met Gln Asn
50 55

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 695
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg
1 5 10 15

Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
20 25 30

Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
35 40 45

Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
50 55 60

Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Gly Leu
65 70 75 80

Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
85 90 95

Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
100 105 110

Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
115 120 125

Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
130 135 140

Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145 150 155 160

Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
165 170 175

Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
180 185 190

Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Cys Asp Val
195 200 205

Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
210 215 220

Val Val Glu Val Ala Glu Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225 230 235 240

Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
245 250 255

Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
260 265 270

Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
275 280 285

Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu
290 295 300

Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys
305 310 315 320

Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg
325 330 335

Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp
340 345 350

Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu
355 360 365

Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala
370 375 380

Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn
385 390 395 400

Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe
405 410 415

Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His
420 425 430

Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala
435 440 445

Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu
450 455 460

Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala
465 470 475 480

Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn
485 490 495

Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser
500 505 510

Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr
515 520 525

Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln
530 535 540

Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn
545 550 555 560

Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr
565 570 575

Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
580 585 590

Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
595 600 605

His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
610 615 620

Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
625 630 635 640

Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
645 650 655

His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
660 665 670

His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
675 680 685

Phe Phe Glu Gln Met Gln Asn
690 695

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2274
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GCTGTGGCAG GGAAGGGGCC ACC ATG GGA TGT ACG CTG AGC GCA GAG GAG 50
Met Gly Cys Thr Leu Ser Ala Glu Glu
1 5

AGA GCC GCC CTC GAG CGG AGC AAG GCG ATT GAG AAA AAC CTC AAA GAA 98
Arg Ala Ala Leu Glu Arg Ser Lys Ala Ile Glu Lys Asn Leu Lys Glu
10 15 20 25

```
GAT GGC ATC AGC GCC GCC AAA GAC GTG AAA TTA CTC CTG CTG GGG GCT        146
Asp Gly Ile Ser Ala Ala Lys Asp Val Lys Leu Leu Leu Leu Gly Ala
                30                  35                  40

GGA GAA TCA GGA AAA AGC ACC ATT GTG AAG CAG ATG AAG ATC ATC CAT        194
Gly Glu Ser Gly Lys Ser Thr Ile Val Lys Gln Met Lys Ile Ile His
            45                  50                  55

GAA GAT GGC TTC TCT GGG GAA GAC GTG AAG CAG TAC AAG CCT GTG GTC        242
Glu Asp Gly Phe Ser Gly Glu Asp Val Lys Gln Tyr Lys Pro Val Val
        60                  65                  70

TAC AGC AAC ACC ATC CAG TCT CTG GCG GCC ATT GTC CGG GCC ATG GAC        290
Tyr Ser Asn Thr Ile Gln Ser Leu Ala Ala Ile Val Arg Ala Met Asp
    75                  80                  85

ACT TTG GGC GTG GAG TAT GGT GAC AAG GAG AGG AAG ACG GAC TCC AAG        338
Thr Leu Gly Val Glu Tyr Gly Asp Lys Glu Arg Lys Thr Asp Ser Lys
90                  95                  100                 105

ATG GTG TGT GAC GTG GTG AGT CGT ATG GAA GAC ACT GAA CCG TTC TCT        386
Met Val Cys Asp Val Val Ser Arg Met Glu Asp Thr Glu Pro Phe Ser
                110                 115                 120

GCA GAA CTT CTT TCT GCC ATG ATG CGA CTC TGG GGC GAC TCG GGG ATC        434
Ala Glu Leu Leu Ser Ala Met Met Arg Leu Trp Gly Asp Ser Gly Ile
            125                 130                 135

CAG GAG TGC TTC AAC CGA TCT CGG GAG TAT CAG CTC AAT GAC TCT GCC        482
Gln Glu Cys Phe Asn Arg Ser Arg Glu Tyr Gln Leu Asn Asp Ser Ala
        140                 145                 150

AAA TAC TAC CTG GAC AGC CTG GAT CGG ATT GGA GCC GGT GAC TAC CAG        530
Lys Tyr Tyr Leu Asp Ser Leu Asp Arg Ile Gly Ala Gly Asp Tyr Gln
    155                 160                 165

CCC ACT GAG CAG GAC ATC CTC CGA ACC AGA GTC AAA ACA ACT GGC ATC        578
Pro Thr Glu Gln Asp Ile Leu Arg Thr Arg Val Lys Thr Thr Gly Ile
170                 175                 180                 185

GTA GAA ACC CAC TTC ACC TTC AAG AAC CTC CAC TTC AGG CTG TTT GAC        626
Val Glu Thr His Phe Thr Phe Lys Asn Leu His Phe Arg Leu Phe Asp
                190                 195                 200

GTC GGG GGC CAG CGA TCT GAA CGC AAG AAG TGG ATC CAC TGC TTT GAG        674
Val Gly Gly Gln Arg Ser Glu Arg Lys Lys Trp Ile His Cys Phe Glu
            205                 210                 215

GAT GTC ACG GCC ATC ATC TTC TGT GTC GCA CTC AGC GGC TAT GAC CAG        722
Asp Val Thr Ala Ile Ile Phe Cys Val Ala Leu Ser Gly Tyr Asp Gln
        220                 225                 230

GTG CTC CAC GAG GAC GAA ACC ACG AAC CGC ATG CAC GAA TCC CTG AAG        770
Val Leu His Glu Asp Glu Thr Thr Asn Arg Met His Glu Ser Leu Lys
    235                 240                 245

CTC TTC GAC AGC ATC TGC AAC AAC AAG TGG TTC ACA GAC ACA TCT ATT        818
Leu Phe Asp Ser Ile Cys Asn Asn Lys Trp Phe Thr Asp Thr Ser Ile
250                 255                 260                 265

ATC CTG TTT CTC AAC AAG AAG GAC ATA TTT GAG GAG AAG ATC AAG AAG        866
Ile Leu Phe Leu Asn Lys Lys Asp Ile Phe Glu Glu Lys Ile Lys Lys
                270                 275                 280
```

TCC CCA CTC ACC ATC TGC TTT CCT GAA TAC ACA CGC CCC AGT GCC TTC 914
Ser Pro Leu Thr Ile Cys Phe Pro Glu Tyr Thr Gly Pro Ser Ala Phe
285 290 295

ACA GAA GCT GTG GCT CAC ATC CAA GGG CAG TAT GAG AGT AAG AAT AAG 962
Thr Glu Ala Val Ala His Ile Gln Gly Gln Tyr Glu Ser Lys Asn Lys
300 305 310

TCA GCT CAC AAG GAA GTC TAC AGC CAT GTC ACC TGT GCC ACG GAC ACC 1010
Ser Ala His Lys Glu Val Tyr Ser His Val Thr Cys Ala Thr Asp Thr
315 320 325

AAC AAC ATC CAA TTC GTC TTT GAT GCC GTG ACA GAT GTC ATC ATC GCC 1058
Asn Asn Ile Gln Phe Val Phe Asp Ala Val Thr Asp Val Ile Ile Ala
330 335 340 345

AAA AAC CTA CGG GGC TGT GGA CTC TAC TGAGCCCTGG CCTCCTACCC 1105
Lys Asn Leu Arg Gly Cys Gly Leu Tyr
350

AGCCTGCCAC TCACTCCTCC CCTGGACCCA GAGCTCTGTC ACTGCTCAGA TGCCCTGTTA 1165

ACTGAAGAAA ACCTGGAGGC TAGCCTTGGG GGCAGGAGGA GGCATCCTTT GAGCATCCCC 1225

ACCCCACCCA ACTTCAGCCT CGTGACACGT GGGAACAGGG TTGGGCAGAG GTGTGGAACA 1285

GCACAAGGCC AGAGACCACG GCATGCCACT TGGGTGCTGC TCACTGGTCA GCTGTGTGTC 1345

TTACACAGAG GCCGAGTGGG CAACACTGCC ATCTGATTCA GAATGGGCAT GCCCTGTCCT 1405

CTGTACCTCT TGTTCAGTGT CCTGGTTTCT CTTCCACCTT GGTGATAGGA TGGCTGGCAG 1465

GAAGGCCCCA TGGAAGGTGC TGCTTGATTA GGGGATAGTC GATGGCATCT CTCAGCAGTC 1525

CTCAGGGTCT GTTTGGTAGA GGGTGGTTTC GTCGACAAAA GCCAACATGG AATCAGGCCA 1585

CTTTTGGGGC GCAAAGACTC AGACTTTGGG GACGGGTTCC CTCCTCCTTC ACTTTGGATC 1645

TTGGCCCCTC TCTGGTCATC TTCCCTTGCC CTTGGGCTCC CCAGGATACT CAGCCCTGAC 1705

TCCCATGGGG TTGGGAATAT TCCTTAAGAC TGGCTGACTG CAAAGGTCAC CGATGGAGAA 1765

ACATCCCTGT GCTACAGAAT TGGGGGTGGG ACAGCTGAGG GGGCAGGCGG CTCTTTCCTG 1825

ATAGTTGATG ACAAGCCCTG AGAATGCCAT CTGCTGGCTC CACTCACACG GGCTCAACTG 1885

TCCTGGGTGA TAGTGACTTG CCAGGCCACA GGCTGCAGGT CACAGACAGA GCAGGCAAGC 1945

AGCCTTGCAA CTGCAGATTA CTTAGGGAGA AGCATCCTAG CCCCAGCTAA CTTTGGACAG 2005

TCAGCATATG TCCCTGCCAT CCCTAGACAT CTCCAGTCAG CTGGTATCAC AGCCAGTGGT 2065

TCAGACAGGT TTGAATGCTC ATGTGGCAGG GGGCCCGGTA CCCAGCTTTT GTTCCCTTTA 2125

GTGAGGGTTA ATTGCGCGCT TGGGCTAATC ATGGTCATAG CTGTTGGGCG TTGCTGGCGT 2185

TTTTCCATAG GCTCCGCCCC CTGACGAGAT CACAAAAATC GACGCTCAAG TCAGAGGTGG 2245

CGAAACCGAC AGACTATAAG ATACCAGGC 2274

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

```
Asp Val Gly Gly Gln Arg Ser Glu Arg Lys Lys Trp Ile His Cys Phe
 1               5                  10                  15

Glu Asp
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

```
Thr Ser Ile Ile Leu Phe Leu Asn Lys Lys Asp Leu
 1               5                  10
```

CLAIMS

1. A method of identifying a therapeutic useful for treating or preventing the symptoms of Alzheimer's disease, which method includes the steps of

contacting (a) a first molecule comprising the couplone portion (SEQ ID NO: 1) of amyloid precursor protein (APP) with (b) a second molecule comprising an APP-associating region of G_o (SEQ ID NOs: 3, 4, or 5), in the presence of a candidate compound; and

determining whether said candidate compound interferes with the association of said first and second molecules, said interference being an indication that said candidate compound is a therapeutic useful for treating Alzheimer's disease.

2. The method of claim 1, wherein said determining step is accomplished by

immmunoprecipitating said first molecule with an antibody specific for APP; and

detecting the presence or amount of said second molecule which co-precipitates with said first molecule.

3. The method of claim 1, wherein said determining step is accomplished by

immunoprecipitating said second molecule with an antibody specific for G_o; and

detecting the presence or amount of said first molecule which co-precipitates with said second molecule.

4. The method of claim 1, wherein said first molecule comprises the portion of APP_{695} from residues 649 to 695 (SEQ ID NO: 6).

5. The method of claim 1, wherein said first molecule comprises the portion of APP_{695} from residues 639 to 648 (SEQ ID NO: 7).

6. The method of claim 1, wherein said first molecule comprises the portion of APP_{695} from residues 640 to 695 (SEQ ID NO: 26).

7. The method of claim 6, wherein said first molecule comprises essentially all of APP_{695} (SEQ ID NO: 27).

8. The method of claim 1, wherein said second molecule comprises the GTP-binding region of G_o (SEQ ID NO: 10).

9. The method of claim 8, wherein said second molecule comprises essentially all of G_o (SEQ ID NO: 2).

10. A method of assaying for a therapeutic useful for treating Alzheimer's disease, which method includes the steps of

contacting (a) a first molecule comprising the couplone region of APP (SEQ ID NO: 1) with (b) a second molecule comprising an APP-associating region of G_o (SEQ ID NO: 3, 4, or 5), in the presence of a candidate compound; and

determining whether said candidate compound interferes with the activation of said second molecule by said first molecule, said interference being an indication that said candidate compound is a therapeutic useful for treating Alzheimer's disease.

11. The method of claim 10, wherein said determining step is accomplished by

contacting said second molecule with a substrate comprising GTP or an analog of GTP; and

detecting or measuring the binding of said substrate to said second molecule, wherein said binding is evidence of said activation of said second molecule by said first molecule.

12. The method of claim 1, wherein said contacting step is carried out at a Mg^{2+} concentration between $1x10^{-7}$ and $1x10^{-2}$ M.

13. The method of claim 10, wherein said contacting step is carried out at a Mg^{2+} concentration between $1x10^{-7}$ and $1x10^{-2}$ M.

14. The method of claim 1, wherein said contacting step is carried out in a cell-free system.

15. The method of claim 10, wherein said contacting step is carried out in a cell-free system.

16. A system for screening candidate Alzheimer's disease therapeutics, which system comprises

a first polypeptide comprising a sequence essentially identical to that of peptide 20 (SEQ ID NO: 1);

a second polypeptide comprising a sequence essentially identical to the anticouplone sequence of G_o (SEQ ID NO: 3); and

a means for detecting either (a) the association of said first polypeptide with said second polypeptide, or (b) the activation of said second polypeptide by said first polypeptide.

17. A cell-free system for screening candidate Alzheimer's disease therapeutics, which system comprises

a first polypeptide comprising a sequence essentially identical to that of peptide 20 (SEQ ID NO: 1); and

a second polypeptide comprising a sequence essentially identical to the anticouplone sequence of G_o (SEQ ID NO: 3).

18. The system of claim 17, wherein said first polypeptide is anchored to a solid material or is in a phospholipid vesicle.

19. The system of claim 17, wherein said second polypeptide further comprises residues 1 to 3 (SEQ ID NO: 4) and 19 to 36 (SEQ ID NO: 5) of G_o.

20. The system of claim 19, wherein said second polypeptide comprises G_o1 or G_o2.

21. A method for diminishing the activation of G_o in a neuronal cell by treating the cell with a compound which blocks association of G_o with the cytoplasmic tail of APP.

22. The method of claim 21, wherein the compound is a peptide fragment of G_o or of the cytoplasmic tail of APP.

23. The method of claim 21, wherein said cell is within an animal.

24. The method of claim 23, wherein said animal is a human.

25. A method for preventing or treating Alzheimer's disease in a patient, comprising treating the patient with a compound which blocks association of G_o with the cytoplasmic tail of APP.

26. A method for preventing or treating Alzheimer's disease in a patient, comprising treating the patient with a compound which inhibits activation of neuronal G_o by the cytoplasmic tail of APP.

27. A peptide having less than 50 amino acids and comprising the sequence of peptide 20 (SEQ ID NO: 1).

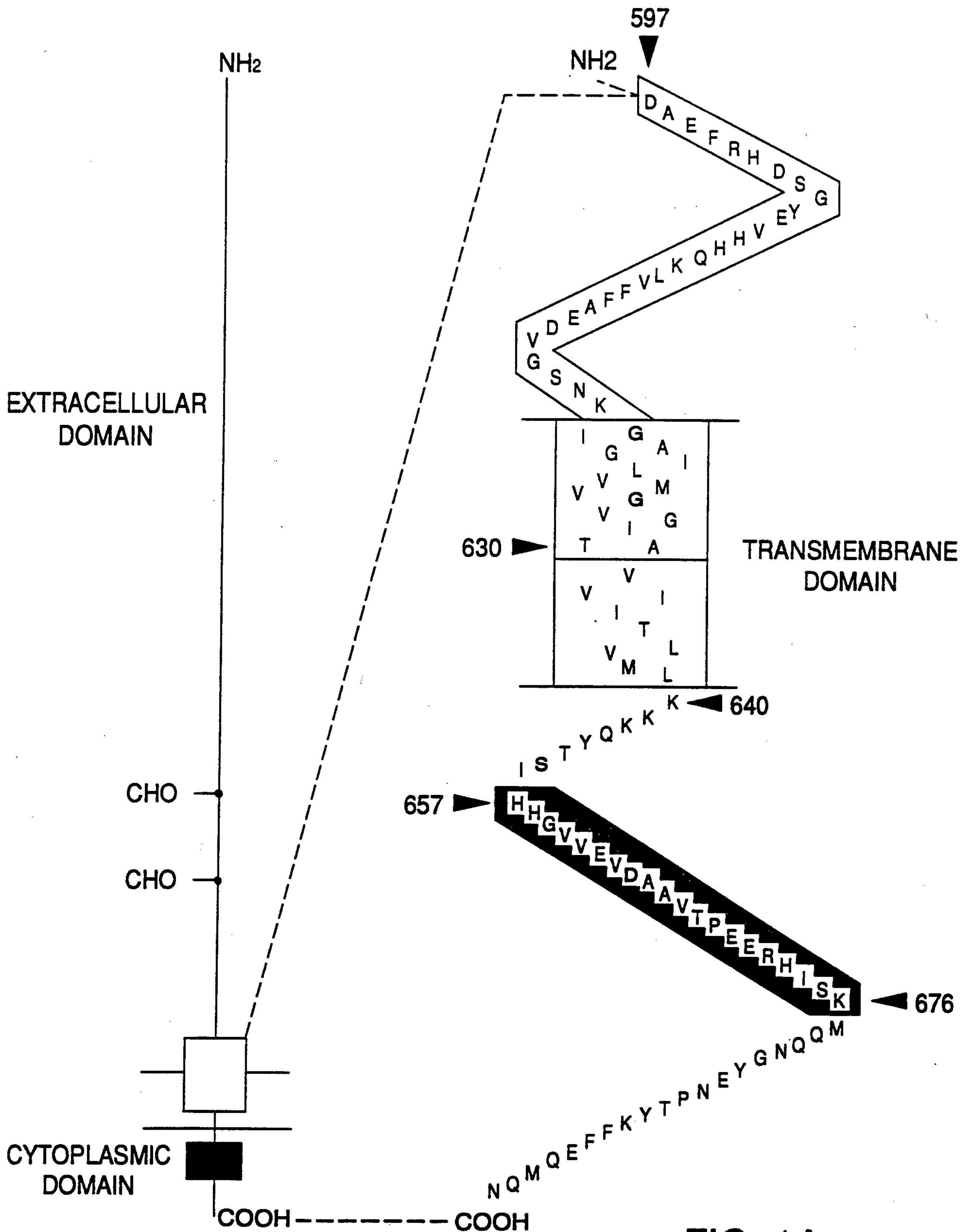
28. A therapeutic composition comprising the peptide of claim 27 and a pharmaceutically acceptable carrier.

29. A method for identifying a ligand for which APP is a receptor, which method includes the steps of

providing an APP molecule and a G_o molecule;

contacting a candidate compound with the extracellular domain of said APP molecule, the cytoplasmic tail of said APP molecule being accessible to said G_o molecule, and

detecting either (a) association of said G_o molecule with said APP molecule, or (b) activation of said G_o molecule by said APP molecule, said association or activation being evidence that said candidate compound is a ligand of APP.



597
NH2
NH2
DAEFRHDSG
YEVHHQKLVFFAEDV
GSNK
IGAI
GLV
VVGM
VIG
TA
630
TRANSMEMBRANE DOMAIN
VVI
IT
VML
L
K
640
ISTYQKK
657
HHGVVEVDAAVTPEERHISK
676
MQNGYENPTYKFFEQMQN
EXTRACELLULAR DOMAIN
CHO
CHO
CYTOPLASMIC DOMAIN
COOH
COOH

FIG. 1A

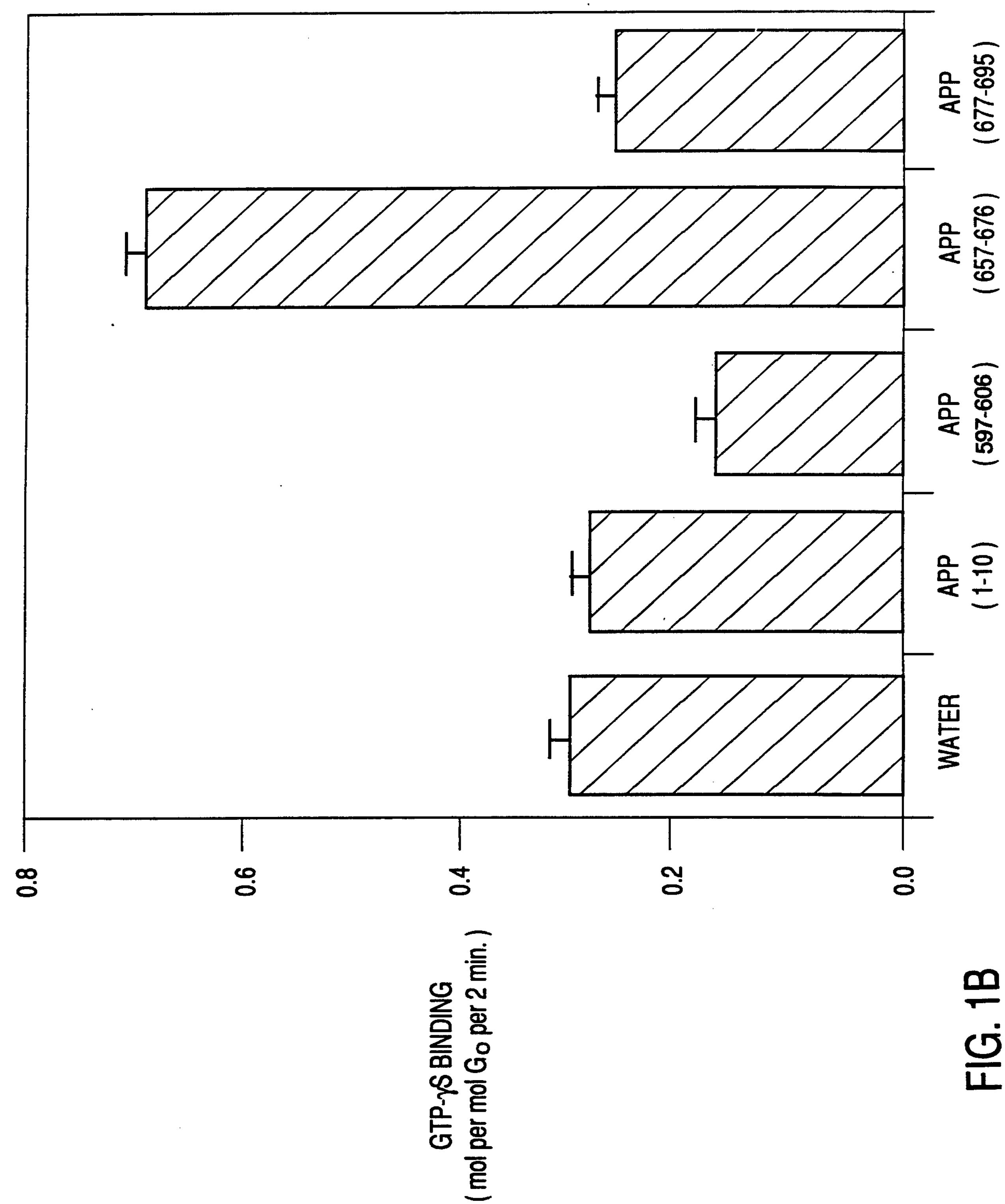

GTP-γS BINDING
(mol per mol G_O per 2 min.)
0.8
0.6
0.4
0.2
0.0
WATER
APP
(1-10)
APP
(597-606)
APP
(657-676)
APP
(677-695)

FIG. 1B

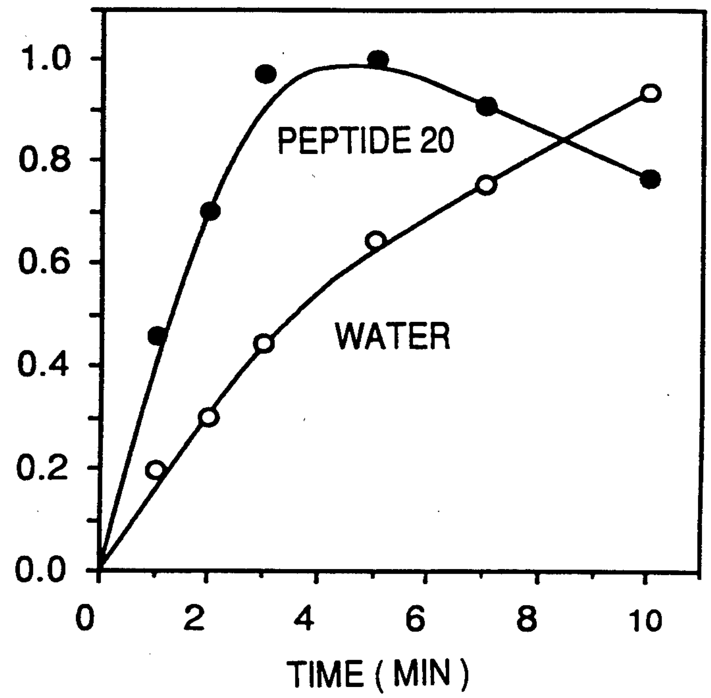

GTP- γ S BOUND
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
PEPTIDE 20
WATER
TIME (MIN)

FIG. 1C

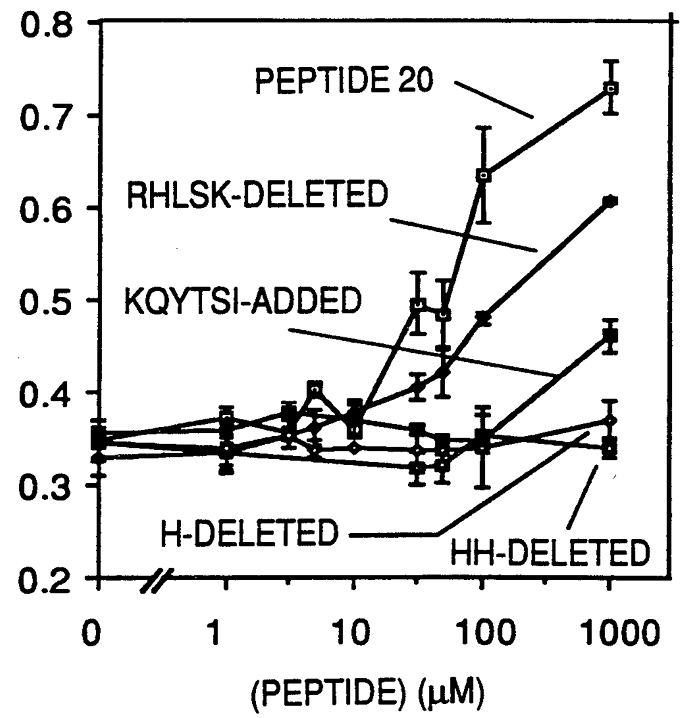

GTP-γS BINDING
(mol per mol Go per 2 min.)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0
1
10
100
1000
PEPTIDE 20
RHLSK-DELETED
KQYTSI-ADDED
H-DELETED
HH-DELETED
(PEPTIDE) (μM)

FIG. 1D

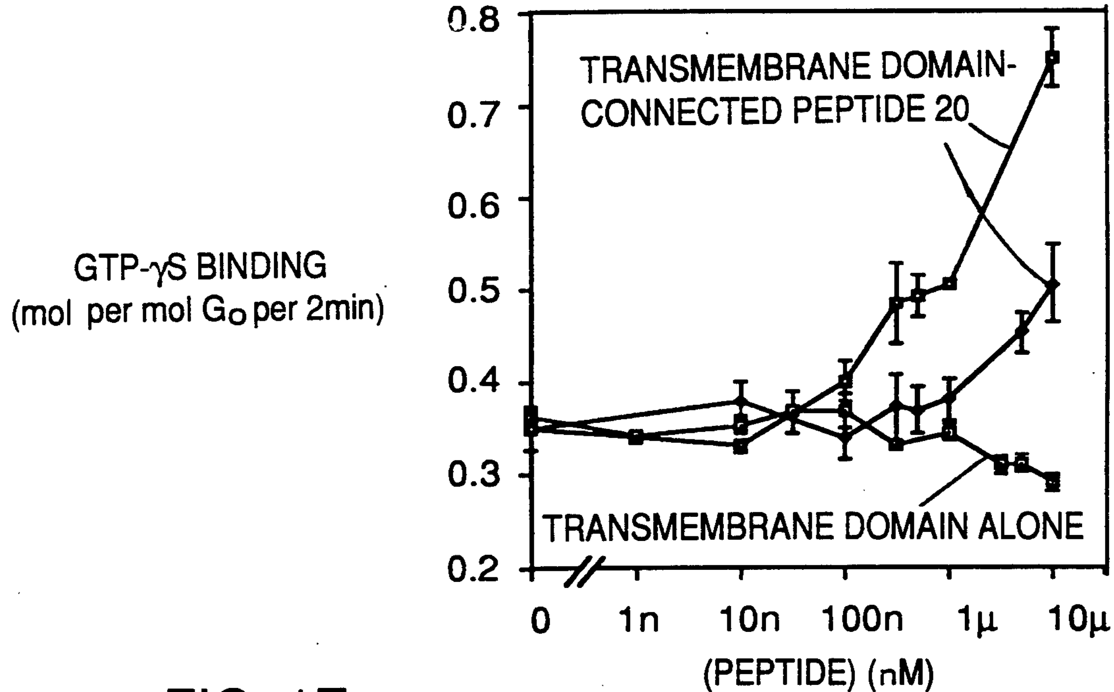

TRANSMEMBRANE DOMAIN-
CONNECTED PEPTIDE 20
TRANSMEMBRANE DOMAIN ALONE
GTP-γS BINDING
(mol per mol Go per 2min)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0
1n
10n
100n
1μ
10μ
(PEPTIDE) (nM)

FIG. 1E

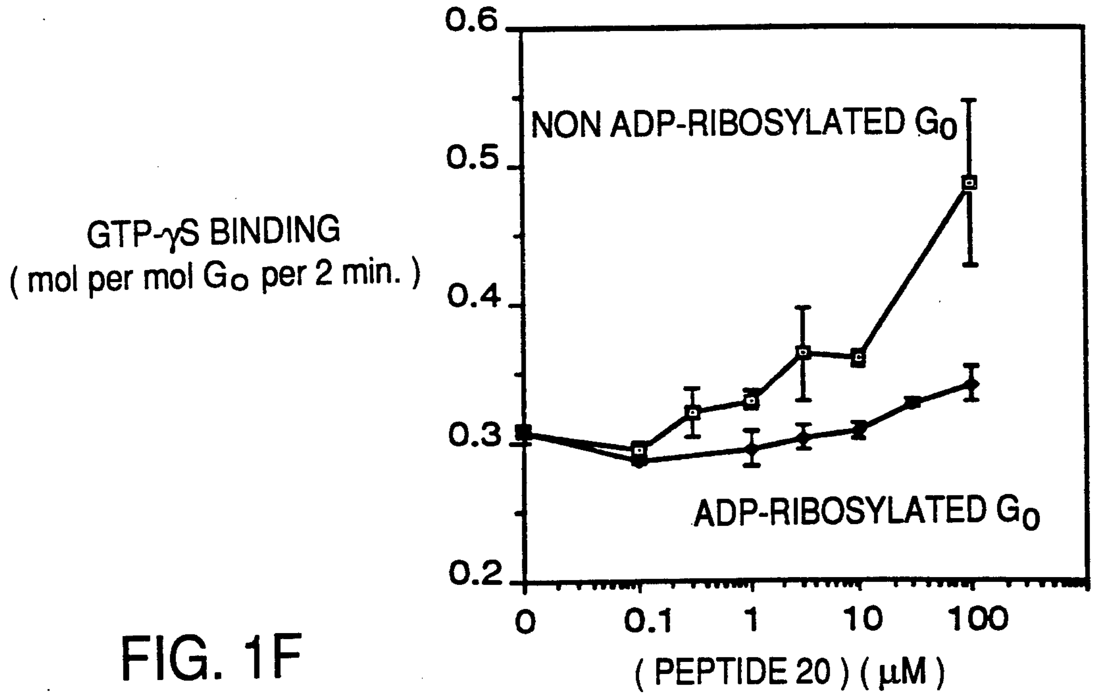

NON ADP-RIBOSYLATED Go
ADP-RIBOSYLATED Go
GTP-γS BINDING
(mol per mol Go per 2 min.)
0.6
0.5
0.4
0.3
0.2
0
0.1
1
10
100
(PEPTIDE 20) (μM)

FIG. 1F

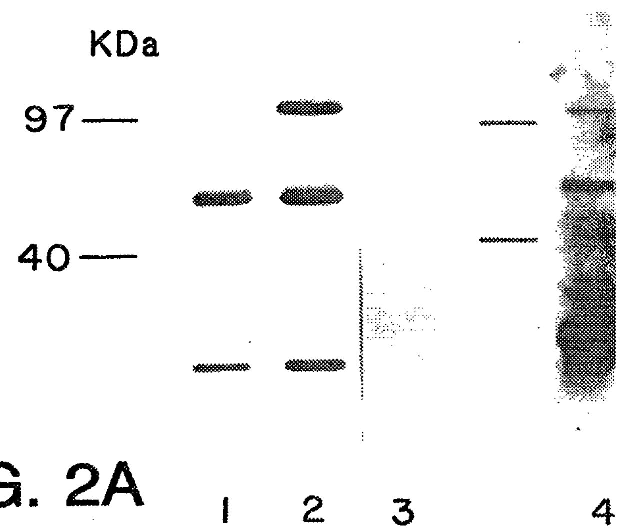


FIG. 2A

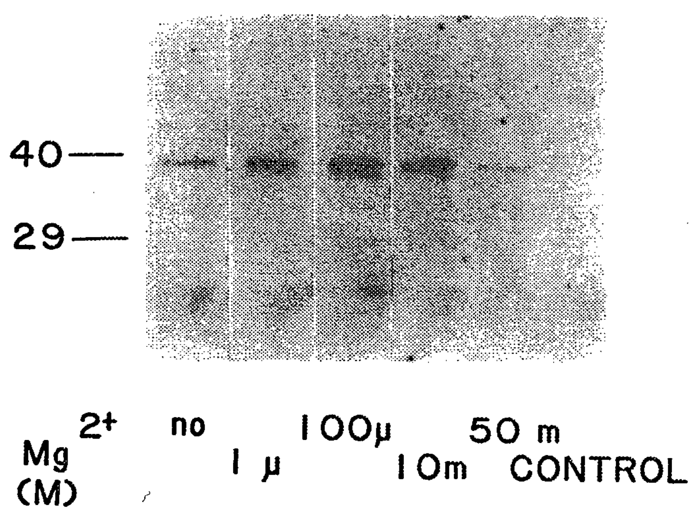


FIG. 2C

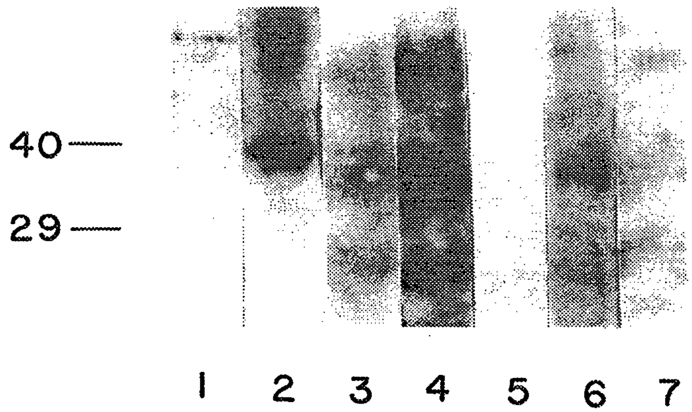

40
29
1 2 3 4 5 6 7

FIG. 2B

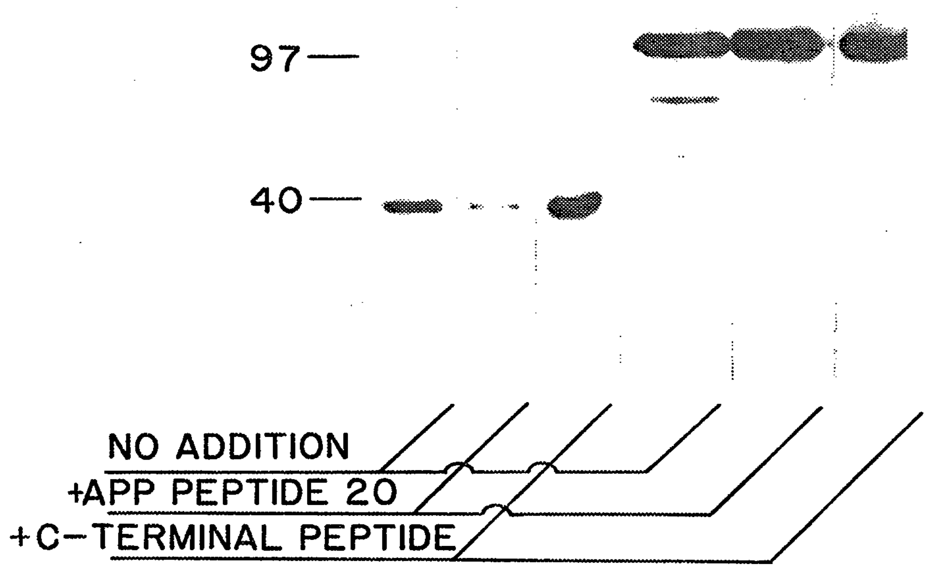

97
40
NO ADDITION
+APP PEPTIDE 20
+C-TERMINAL PEPTIDE

FIG. 2D

FIG. 3A

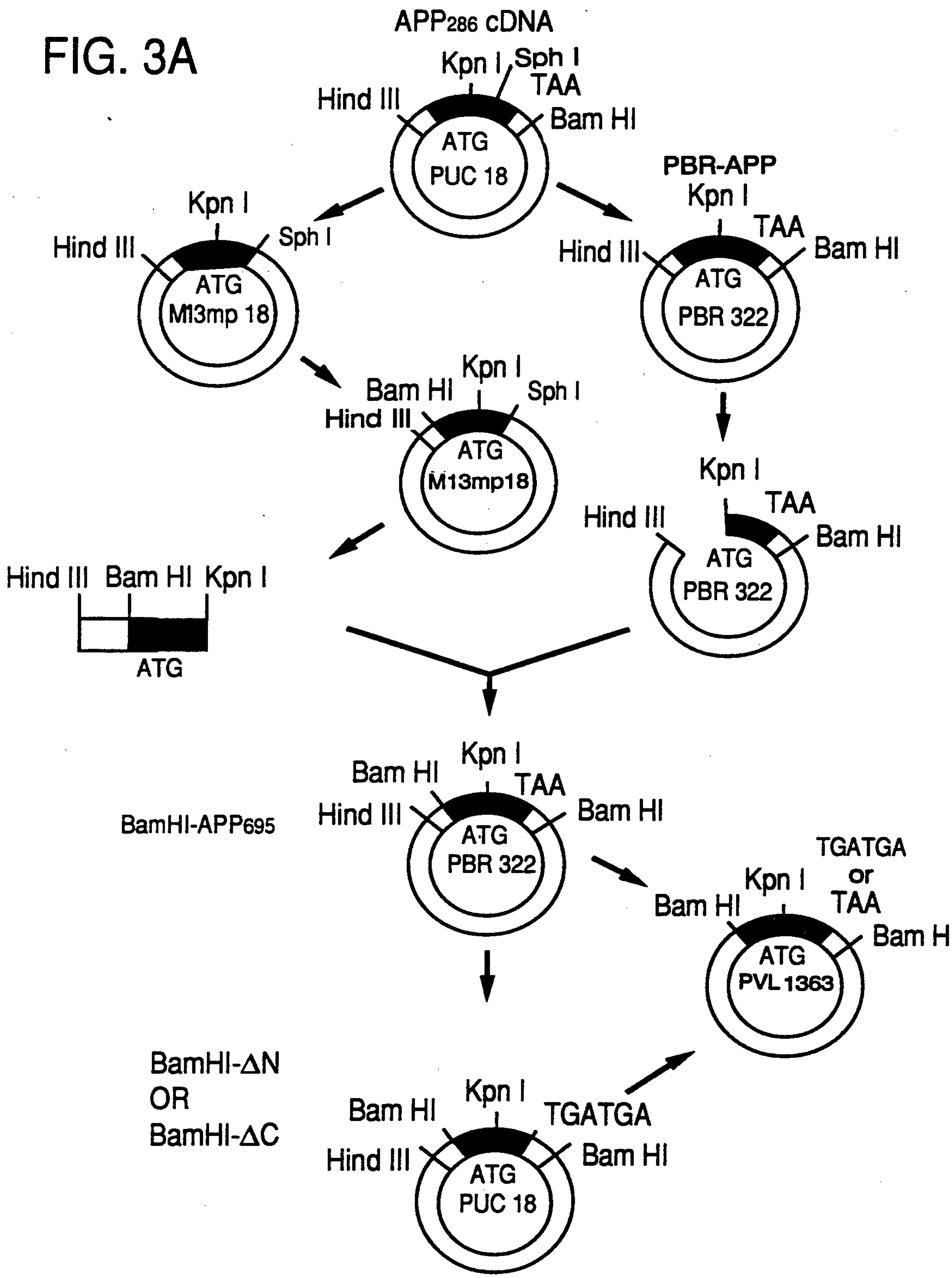
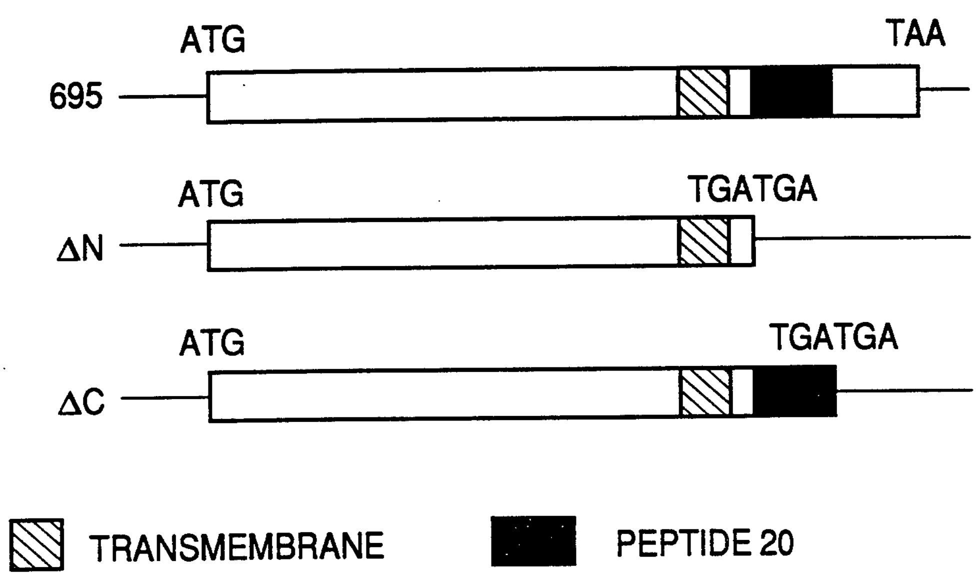

APP286 cDNA
Kpn I
Sph I
TAA
Hind III
Bam HI
ATG
PUC 18
PBR-APP
Kpn I
Hind III
Sph I
ATG
M13mp 18
Kpn I
TAA
Hind III
Bam HI
ATG
PBR 322
Kpn I
Bam HI
Sph I
Hind III
ATG
M13mp18
Kpn I
TAA
Hind III
Bam HI
ATG
PBR 322
Hind III Bam HI Kpn I
ATG
Kpn I
Bam HI
TAA
BamHI-APP695
Hind III
Bam HI
ATG
PBR 322
TGATGA
or
TAA
Kpn I
Bam HI
Bam HI
ATG
PVL 1363
BamHI-ΔN
OR
BamHI-ΔC
Kpn I
Bam HI
TGATGA
Hind III
Bam HI
ATG
PUC 18

FIG. 3B


ATG
TAA
695
ATG
TGATGA
ΔN
ATG
TGATGA
ΔC
TRANSMEMBRANE
PEPTIDE 20

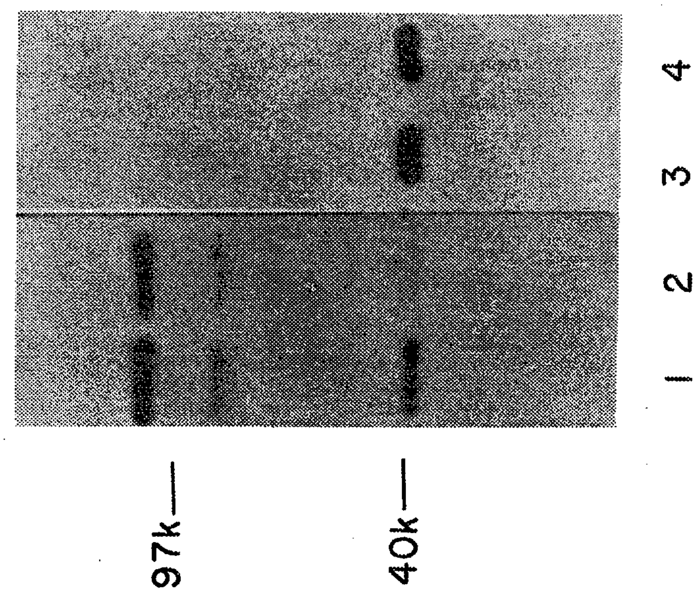


FIG. 3E

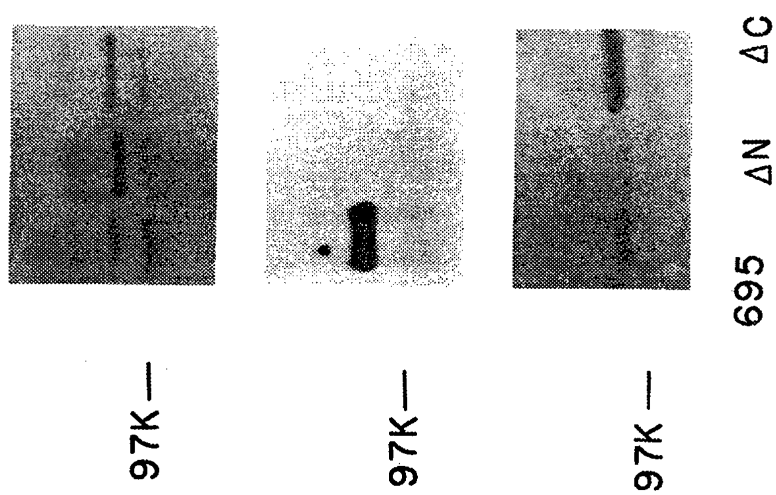


FIG. 3C

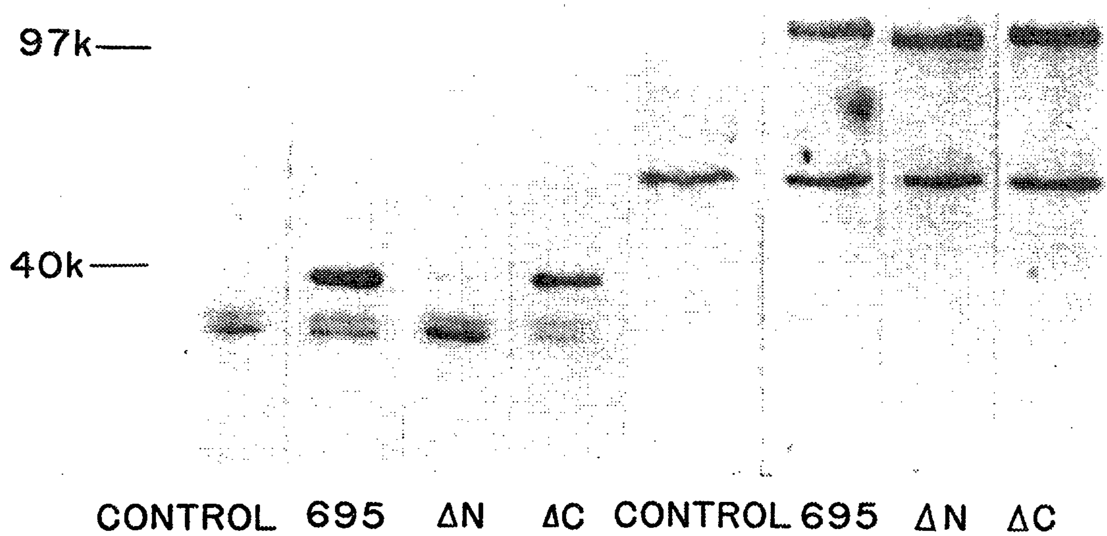


FIG. 3D

```
TGTGGCAGGG AAGGGGCCAC C ATG GGA TGT ACG CTG AGC GCA GAG GAG AGA    51
                        Met Gly Cys Thr Leu Ser Ala Glu Glu Arg
                         1               5                  10

GCC GCC CTC GAG CGG AGC AAG GCG ATT GAG AAA AAC CTA AAA GAA GAT    99
Ala Ala Leu Glu Arg Ser Lys Ala Ile Glu Lys Asn Leu Lys Glu Asp
                15                  20                  25

GGC ATC AGC GCC GCC AAA GAC GTG AAA TTA CTC CTG CTG GGG GCT GGA   147
Gly Ile Ser Ala Ala Lys Asp Val Lys Leu Leu Leu Leu Gly Ala Gly
            30                  35                  40

GAA TCA GGA AAA AGC ACC ATT GTG AAG CAG ATG AAG ATC ATC CAT GAA   195
Glu Ser Gly Lys Ser Thr Ile Val Lys Gln Met Lys Ile Ile His Glu
        45                  50                  55

GAT GGC TTC TCT GGG GAA GAC GTG AAG CAG TAC AAG CCT GTG GTC TAC   243
Asp Gly Phe Ser Gly Glu Asp Val Lys Gln Tyr Lys Pro Val Val Tyr
    60                  65                  70

AGC AAC ACC ATC CAG TCT CTG GCG GCC ATT GTC CGG GCC ATG GAC ACT   291
Ser Asn Thr Ile Gln Ser Leu Ala Ala Ile Val Arg Ala Met Asp Thr
 75                  80                  85                  90

TTG GGC GTG GAG TAT GGT GAC AAG GAG AGG AAG ACG GAC TCC AAG ATG   339
Leu Gly Val Glu Tyr Gly Asp Lys Glu Arg Lys Thr Asp Ser Lys Met
                95                 100                 105

GTG TGT GAC GTG GTG AGT CGT ATG GAA GAC ACT GAA CCG TTC TCT GCA   387
Val Cys Asp Val Val Ser Arg Met Glu Asp Thr Glu Pro Phe Ser Ala
            110                 115                 120
```

FIG. 4A-1

```
GAA CTT CTT TCT GCC ATG ATG CGA CTC TGG GGC GAC TCG GGG ATC CAG      435
Glu Leu Leu Ser Ala Met Met Arg Leu Trp Gly Asp Ser Gly Ile Gln
        125                 130                 135

GAG TGC TTC AAC CGA TCT CGG GAG TAT CAG CTC AAT GAC TCT GCC AAA      483
Glu Cys Phe Asn Arg Ser Arg Glu Tyr Gln Leu Asn Asp Ser Ala Lys
    140                 145                 150

TAC TAC CTG GAC AGC CTG GAT CGG ATT GGA GCC GGT GAC TAC CAG CCC      531
Tyr Tyr Leu Asp Ser Leu Asp Arg Ile Gly Ala Gly Asp Tyr Gln Pro
155                 160                 165                 170

ACT GAG CAG GAC ATC CTC CGA ACC AGA GTC AAA ACA ACT GGC ATC GTA      579
Thr Glu Gln Asp Ile Leu Arg Thr Arg Val Lys Thr Thr Gly Ile Val
                175                 180                 185

GAA ACC CAC TTC ACC TTC AAG AAC CTC CAC TTC AGG CTG TTT GAC GTC      627
Glu Thr His Phe Thr Phe Lys Asn Leu His Phe Arg Leu Phe Asp Val
            190                 195                 200

GGG GGC CAG CGA TCT GAA CGC AAG AAG TGG ATC CAC TGC TTT GAG GAT      675
Gly Gly Gln Arg Ser Glu Arg Lys Lys Trp Ile His Cys Phe Glu Asp
        205                 210                 215

GTC ACG GCC ATC ATC TTC TGT GTC GCA CTC AGC GGC TAT GAC CAG GTG      723
Val Thr Ala Ile Ile Phe Cys Val Ala Leu Ser Gly Tyr Asp Gln Val
    220                 225                 230

CTC CAC GAG GAC GAA ACC ACG AAC CGC ATG CAC GAG TCT CTC ATG CTC      771
Leu His Glu Asp Glu Thr Thr Asn Arg Met His Glu Ser Leu Met Leu
235                 240                 245                 250
```

FIG. 4A-2

```
TTC GAC TCC ATC TGT AAC AAC AAG TTT TTC ATT GAT ACC TCC ATC ATC      819
Phe Asp Ser Ile Cys Asn Asn Lys Phe Phe Ile Asp Thr Ser Ile Ile
                255                 260                 265

CTC TTC CTC AAC AAG AAA GAC CTC TTT GGC GAG AAG ATT AAG AAG TCA      867
Leu Phe Leu Asn Lys Lys Asp Leu Phe Gly Glu Lys Ile Lys Lys Ser
            270                 275                 280

CCC TTG ACC ATC TGC TTT CCC GAA TAC CCA GGC TCC AAC ACC TAT GAA      915
Pro Leu Thr Ile Cys Phe Pro Glu Tyr Pro Gly Ser Asn Thr Tyr Glu
        285                 290                 295

GAT GCA GCT GCC TAC ATC CAA ACA CAG TTT GAA AGC AAA AAC CGC TCA      963
Asp Ala Ala Ala Tyr Ile Gln Thr Gln Phe Glu Ser Lys Asn Arg Ser
    300                 305                 310

CCC AAC AAA GAA ATT TAC TGT CAC ATG ACT TGT GCC ACA GAC ACG AAT     1011
Pro Asn Lys Glu Ile Tyr Cys His Met Thr Cys Ala Thr Asp Thr Asn
315                 320                 325                 330

AAT ATC CAG GTG GTA TTC GAC GCC GTC ACC GAC ATC ATC ATT GCC AAC     1059
Asn Ile Gln Val Val Phe Asp Ala Val Thr Asp Ile Ile Ile Ala Asn
                335                 340                 345

AAT CTC CGG GGC TGC GGC TTG TAC TGACCTCTTG TCCTGTATAG CAACCTATTT    1113
Asn Leu Arg Gly Cys Gly Leu Tyr
            350
```

FIG. 4A-3

```
GACTGCTTCA TGGACTCTTT GCTGTTGATG TTGATCTCCT GGTAGCATGA CCTTTGGCCT 1173
TTGTAAGACA CACAGCCTTT CTGTACCAAG CCCCTGTCTA ACCTACGACC CCAGAGTGAC 1233
TGACGGCTGT GTATTTCTGT AGAATGCTGT AGAATACAGT TTTAGTTGAG TCTTTACATT 1293
TAGAACTTGA AAGGATTTTA AAAAACAAAA CAAAAACCAT TTCTCATGTG CTTTGTAGCT 1353
TTAAAAGAAA AAAGGAAAAC TCACCATTTA ATCCATATTT CCTTTTTATT TTGAAGTTTA 1413
AAAAAAAAAT GTCTGTACCC ACACCCTCCC CCTTCCCCAC CTCAGCAGAA CTGGGGCTGG 1473
CACACAGAGG CAGTGCTGGG CCTGGCGCCT CCCAGGGCTT CTGTGCAGCC CATGGCTGGT 1533
GGGAACATGT CAGGCTAGTC TGTCTAGAAG GCCACTGGCC ACTGTACCCA CCCTTCCCCA 1593
TGCCTGTGGG CTGCCCAGAC ACCTCATATA CCACCAGGCA GTGGCAGCTC CGCCCTGCTC 1653
AGCCATGCGA CTCCAAACAC ACTCAAAGTT TGCGTAGAAA AAGCACAGCT CTGGCAGGGG 1713
TAGCTGCCAC AGACAACGCT CATCACCTAT AGAAATCCAG CCCTATAGAA GCAATTCACC 1773
CAGCCCCTTC CTACACTCCC TTTGTGTTGT TAACTTTTTG GTTTTTCTGG TCCTAGTGAG 1833
TGCCTCCCAT GCATACCTGA CCAGCTCTGC CAGTGTCTGG GGTCTGGGGA ACAGGGGTTG 1893
TGTGGTTTGG TTTTTGG                                                1910
```

FIG. 4A-4

```
GCTGTGGCAG GGAAGGGGCC ACC ATG GGA TGT ACG CTG AGC GCA GAG GAG        50
                          Met Gly Cys Thr Leu Ser Ala Glu Glu
                           1               5

AGA GCC GCC CTC GAG CGG AGC AAG GCG ATT GAG AAA AAC CTC AAA GAA      98
Arg Ala Ala Leu Glu Arg Ser Lys Ala Ile Glu Lys Asn Leu Lys Glu
 10                  15                  20                  25

GAT GGC ATC AGC GCC GCC AAA GAC GTG AAA TTA CTC CTG CTG GGG GCT     146
Asp Gly Ile Ser Ala Ala Lys Asp Val Lys Leu Leu Leu Leu Gly Ala
                 30                  35                  40

GGA GAA TCA GGA AAA AGC ACC ATT GTG AAG CAG ATG AAG ATC ATC CAT     194
Gly Glu Ser Gly Lys Ser Thr Ile Val Lys Gln Met Lys Ile Ile His
             45                  50                  55

GAA GAT GGC TTC TCT GGG GAA GAC GTG AAG CAG TAC AAG CCT GTG GTC     242
Glu Asp Gly Phe Ser Gly Glu Asp Val Lys Gln Tyr Lys Pro Val Val
         60                  65                  70

TAC AGC AAC ACC ATC CAG TCT CTG GCG GCC ATT GTC CGG GCC ATG GAC     290
Tyr Ser Asn Thr Ile Gln Ser Leu Ala Ala Ile Val Arg Ala Met Asp
     75                  80                  85

ACT TTG GGC GTG GAG TAT GGT GAC AAG GAG AGG AAG ACG GAC TCC AAG     338
Thr Leu Gly Val Glu Tyr Gly Asp Lys Glu Arg Lys Thr Asp Ser Lys
 90                  95                 100                 105

ATG GTG TGT GAC GTG GTG AGT CGT ATG GAA GAC ACT GAA CCG TTC TCT     386
Met Val Cys Asp Val Val Ser Arg Met Glu Asp Thr Glu Pro Phe Ser
                110                 115                 120
```

FIG. 4B-1

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GCA GAA CTT CTT TCT GCC ATG ATG CGA CTC TGG GGC GAC TCG GGG ATC     434
Ala Glu Leu Leu Ser Ala Met Met Arg Leu Trp Gly Asp Ser Gly Ile
            125                 130                 135

CAG GAG TGC TTC AAC CGA TCT CGG GAG TAT CAG CTC AAT GAC TCT GCC     482
Gln Glu Cys Phe Asn Arg Ser Arg Glu Tyr Gln Leu Asn Asp Ser Ala
        140                 145                 150

AAA TAC TAC CTG GAC AGC CTG GAT CGG ATT GGA GCC GGT GAC TAC CAG     530
Lys Tyr Tyr Leu Asp Ser Leu Asp Arg Ile Gly Ala Gly Asp Tyr Gln
    155                 160                 165

CCC ACT GAG CAG GAC ATC CTC CGA ACC AGA GTC AAA ACA ACT GGC ATC     578
Pro Thr Glu Gln Asp Ile Leu Arg Thr Arg Val Lys Thr Thr Gly Ile
170                 175                 180                 185

GTA GAA ACC CAC TTC ACC TTC AAG AAC CTC CAC TTC AGG CTG TTT GAC     626
Val Glu Thr His Phe Thr Phe Lys Asn Leu His Phe Arg Leu Phe Asp
                190                 195                 200

GTC GGG GGC CAG CGA TCT GAA CGC AAG AAG TGG ATC CAC TGC TTT GAG     674
Val Gly Gly Gln Arg Ser Glu Arg Lys Lys Trp Ile His Cys Phe Glu
            205                 210                 215

GAT GTC ACG GCC ATC ATC TTC TGT GTC GCA CTC AGC GGC TAT GAC CAG     722
Asp Val Thr Ala Ile Ile Phe Cys Val Ala Leu Ser Gly Tyr Asp Gln
        220                 225                 230

GTG CTC CAC GAG GAC GAA ACC ACG AAC CGC ATG CAC GAA TCC CTG AAG     770
Val Leu His Glu Asp Glu Thr Thr Asn Arg Met His Glu Ser Leu Lys
    235                 240                 245
```

FIG. 4B-2

```
CTC TTC GAC AGC ATC TGC AAC AAC AAG TGG TTC ACA GAC ACA TCT ATT     818
Leu Phe Asp Ser Ile Cys Asn Asn Lys Trp Phe Thr Asp Thr Ser Ile
250                 255                 260                 265

ATC CTG TTT CTC AAC AAG AAG GAC ATA TTT GAG GAG AAG ATC AAG AAG     866
Ile Leu Phe Leu Asn Lys Lys Asp Ile Phe Glu Glu Lys Ile Lys Lys
                270                 275                 280

TCC CCA CTC ACC ATC TGC TTT CCT GAA TAC ACA CGC CCC AGT GCC TTC     914
Ser Pro Leu Thr Ile Cys Phe Pro Glu Tyr Thr Gly Pro Ser Ala Phe
            285                 290                 295

ACA GAA GCT GTG GCT CAC ATC CAA GGG CAG TAT GAG AGT AAG AAT AAG     962
Thr Glu Ala Val Ala His Ile Gln Gly Gln Tyr Glu Ser Lys Asn Lys
        300                 305                 310

TCA GCT CAC AAG GAA GTC TAC AGC CAT GTC ACC TGT GCC ACG GAC ACC    1010
Ser Ala His Lys Glu Val Tyr Ser His Val Thr Cys Ala Thr Asp Thr
    315                 320                 325

AAC AAC ATC CAA TTC GTC TTT GAT GCC GTG ACA GAT GTC ATC ATC GCC    1058
Asn Asn Ile Gln Phe Val Phe Asp Ala Val Thr Asp Val Ile Ile Ala
330                 335                 340                 345

AAA AAC CTA CGG GGC TGT GGA CTC TAC TGAGCCCTGG CCTCCTACCC          1105
Lys Asn Leu Arg Gly Cys Gly Leu Tyr
                350

AGCCTGCCAC TCACTCCTCC CCTGGACCCA GAGCTCTGTC ACTGCTCAGA TGCCCTGTTA 1165

ACTGAAGAAA ACCTGGAGGC TAGCCTTGGG GGCAGGAGGA GGCATCCTTT GAGCATCCCC 1225

ACCCCACCCA ACTTCAGCCT CGTGACACGT GGGAACAGGG TTGGGCAGAG GTGTGGAACA 1285
```

FIG. 4B-3

GCACAAGGCC AGAGACCACG GCATGCCACT TGGGTGCTGC TCACTGGTCA GCTGTGTGTC 1345
TTACACAGAG GCCGAGTGGG CAACACTGCC ATCTGATTCA GAATGGGCAT GCCCTGTCCT 1405
CTGTACCTCT TGTTCAGTGT CCTGGTTTCT CTTCCACCTT GGTGATAGGA TGGCTGGCAG 1465
GAAGGCCCCA TGGAAGGTGC TGCTTGATTA GGGGATAGTC GATGGCATCT CTCAGCAGTC 1525
CTCAGGGTCT GTTTGGTAGA GGGTGGTTTC GTCGACAAAA GCCAACATGG AATCAGGCCA 1585
CTTTTGGGGC GCAAAGACTC AGACTTTGGG GACGGGTTCC CTCCTCCTTC ACTTTGGATC 1645
TTGGCCCCTC TCTGGTCATC TTCCCTTGCC CTTGGGCTCC CCAGGATACT CAGCCCTGAC 1705
TCCCATGGGG TTGGGAATAT TCCTTAAGAC TGGCTGACTG CAAAGGTCAC CGATGGAGAA 1765
ACATCCCTGT GCTACAGAAT TGGGGGTGGG ACAGCTGAGG GGGCAGGCGG CTCTTTCCTG 1825
ATAGTTGATG ACAAGCCCTG AGAATGCCAT CTGCTGGCTC CACTCACACG GGCTCAACTG 1885
TCCTGGGTGA TAGTGACTTG CCAGGCCACA GGCTGCAGGT CACAGACAGA GCAGGCAAGC 1945
AGCCTTGCAA CTGCAGATTA CTTAGGGAGA AGCATCCTAG CCCCAGCTAA CTTTGGACAG 2005
TCAGCATATG TCCCTGCCAT CCCTAGACAT CTCCAGTCAG CTGGTATCAC AGCCAGTGGT 2065
TCAGACAGGT TTGAATGCTC ATGTGGCAGG GGGCCCGGTA CCCAGCTTTT GTTCCCTTTA 2125
GTGAGGGTTA ATTGCGCGCT TGGGCTAATC ATGGTCATAG CTGTTGGGCG TTGCTGGCGT 2185
TTTTCCATAG GCTCCGCCCC CTGACGAGAT CACAAAAATC GACGCTCAAG TCAGAGGTGG 2245
CGAAACCGAC AGACTATAAG ATACCAGGC 2274

FIG. 4B-4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/01712

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : G01N 33/543; C12Q 1/68; C07K 15/00
US CL : 436/518; 435/6; 530/350
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 436/518, 536; 435/6, 7.2, 7.21; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Dialog

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	Nature, Vol. 362, issued 04 March 1993, Nishimoto et al., "Alzheimer amyloid protein precursor complexes with brain GTP-binding protein Go," pages 75-79, see entire document.	1-20, 27-29

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 APRIL 1994	25 APR 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DONNA C. WORTMAN Jill Warden for Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/01712

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

I. Claims 1-20, 27-29, drawn to a composition and a method of use, Class 436, Subclass 518, and Class 530, subclass 350.

II. Claims 21-26, drawn to a treatment method, Class 512, Subclass 12.

Groups I and II do not share a common special technical feature as represented in PCT Rule 13.2 because they are drawn to completely different methods requiring different process steps for completion. Note that PCT Rule 13.2 does not provide for multiple methods within a single inventive concept.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
   1-20, 27-29

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.